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(71) Applicant (*for all designated States except US*): **THE ROSLIN INSTITUTE** [GB/GB]; Roslin, Midlothian EH25 9PS (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **HALEY, Christopher, Simon** [GB/GB]; 35 Grange Loan, Edinburgh EH9 2ER (GB). **ARCHIBALD, Alan, Langskill** [GB/GB]; 70 Silverknowes View, Edinburgh EH4 5PS (GB).

(74) Agent: **MURGITROYD & COMPANY**; 373 Scotland Street, Glasgow G5 8QA (GB).

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(54) Title: ASSAY

(57) Abstract: The present invention provides a method for determining the predisposition of pigs to boar taint. Boar taint is a strong unpleasant odour given off upon heating or cooking of meat from uncastrated male pigs. Boar taint is associated with elevated levels of skatole, indole and androstenone. There are significant economic losses attributable to current methods of preventing or producing the effect of boar taint. Thus, the identification of animals of the desired genotype allows for the selection against animals with a genetic predisposition to boar taint, this being an attractive, cost effective and humane solution to the boar taint problem. The present invention thus identifies QTL for boar taint and its component traits. In particular said traits are shown to be particularly located on chromosome 6 and 14, and further an important candidate gene mapping to chromosome 14 is also shown.

1 **"Assay"**

2

3 The present invention relates to genetic markers for
4 pigs exhibiting desirable flavour properties. In
5 particular, the present invention provides an assay
6 to screen pigs for boar taint and its associated
7 flavours. Generally pigs having low boar taint
8 levels will be positively selected, but it is also
9 possible to identify animals having unacceptably high
10 boar taint levels.

11

12 Boar taint is a strong perspiration-like, urine-like
13 unpleasant odour given off upon heating or cooking of
14 meat from some entire (uncastrated) male pigs. The
15 off-odours and off-tastes, commonly known as "boar
16 taint", are objectionable to consumers. In the
17 United States carcasses tainted by boar odour are
18 either condemned or subject to restricted use by
19 United States Department of Agriculture meat
20 inspectors. EU law (Council Directive 91/497/EEC,
21 which has been implemented in Britain through the

1 Fresh Meat (Hygiene and Inspection) Regulations 1992)
2 states that animals over 80 kg carcase weight,
3 excluding the head, should be screened for boar
4 taint, but no method is specified.

5
6 The most effective method, to date, for preventing
7 "boar taint" is to castrate (remove the testes of)
8 young male pigs. Castration of young male pigs is
9 widely practised in pig production systems in North
10 America and Europe. However, as outlined below,
11 there are production advantages of using entire male
12 pigs. Entire male pigs are used extensively in pig
13 production in the United Kingdom and also in Denmark,
14 Australia and parts of Spain. Other measures taken
15 to reduce the risk of boar taint include slaughtering
16 entire male pigs at an earlier age than castrated
17 males.

18
19 Pig production systems that involve castration of
20 young male pigs suffer economic losses and other
21 disadvantages. These economic losses are
22 attributable to lost opportunities to access the
23 superior performance, especially feed conversion, of
24 intact males and the inferior nature of carcasses
25 from castrates (barrows) (for example: Allen, P.,
26 Riordan, P.B., Hanrahan, T.J. and Joseph, R.L. 1981.
27 Production and quality of boar and castrate bacon.
28 *Irish J. Sci. Technol.* 5, 93-104; Wood, J.D. and
29 Riley, J.E. 1982. Comparison of boars and castrates
30 for bacon production. 1. Growth data, and carcass

1 and joint composition. *Animal Production* 35, 55-63;
2 Ellis, M., Smith, W.C., Clark, J.B.K. and Innes, N.
3 1983. A comparison of boars, gilts and castrates for
4 bacon manufacture. 1. on farm performance, carcass
5 and meat quality characteristics and weight loss in
6 the preparation of sides for curing. *Animal*
7 *Production* 37, 1-9). If the problem of boar taint
8 were overcome, raising boars rather than castrates
9 would have considerable economic advantages.

10 Although boars and castrates gain weight at
11 equivalent rates, boars produce carcasses containing
12 20-30% less fat. Boars also utilise feed more
13 efficiently than castrates (10% less feed consumed
14 per unit of body weight). Since feed represents the
15 major cost in pig production, raising boars for pork
16 would have significant economic advantages.

17
18 Castration not only produces animals with inferior
19 carcass characteristics and a less efficient feed
20 conversion, but is also bad for the pig's welfare.
21 Adverse animal welfare considerations include the
22 pain associated with castration, the loss of 'normal'
23 behaviour and the risk of infection.

24
25 In conclusion, there is a need for methods to prevent
26 or determine predisposition to boar taint, that do
27 not require castration of young pigs.

28

29 Boar taint

30

1 Boar taint is associated with elevated levels of
2 androstenone (5 -androst-16-en-3-one), indole and
3 skatole (3-methyl-1H-indole) (Patterson, 1968;
4 Bonneau, 1982; see also Claus et al. 1994.
5 Physiological aspects of androstenone and skatole
6 formation in the boar - a review with experimental
7 data. Meat Science 38, 289-305).

8

9 Androstenone gives a urine or perspiration-like
10 odour, whilst indole and skatole give a camphor-like
11 odour. Levels of androstenone and skatole are each
12 increased in non-castrated boars, although the reason
13 for increased skatole levels has not been
14 established. Additionally the formation of
15 androstenone and skatole appears to be independent
16 although the degradation of these compounds is
17 currently believed to follow similar pathways and may
18 each involve cytochrome P450s. There remains debate
19 concerning the relative importance of androstenone
20 and skatole in contributing to boar taint, and in
21 certain studies emphasis has been placed onto
22 androstenone (see WO 98/41861 and WO 99/18192).

23

24 Methods that address the variation in levels of both
25 compounds would be particularly useful for breeding
26 male slaughter pigs.

27

28 The 16-androstene steroids, such as 5 -androst-16-en-
29 3-one (androstenone), are produced in the Leydig
30 cells of the testis and passed into the bloodstream

1 (Bonneau, 1982). Due to their hydrophobic nature,
2 16-androstene steroids are subsequently absorbed by
3 fatty tissues.

4
5 Skatole (3-methyl-indole) is produced by the
6 breakdown of tryptophan by bacteria in the hind gut
7 of pigs and other animals (see Moss et al., "Boar
8 taint: the role of skatole", Meat Focus
9 International, October 1992; and Babol et al., "Boar
10 taint in entire male pigs", EAAP Publication No 92).
11 Skatole is absorbed into the bloodstream and
12 deposited in fatty tissues.

13
14 Methods for the identification and production of
15 swine with reduced boar taint are described In
16 WO 99/18192. The method of WO 99/18192 is concerned
17 with androstenone production and in particular the
18 predicted impact of specific natural or
19 experimentally-induced mutations or polymorphisms in
20 the porcine CYP17 gene which encodes cytochrome
21 P450c17. Cytochrome P450c17 is required for
22 production of androstenone. A method for determining
23 predisposition to boar taint is disclosed in WO
24 98/41861. The method of WO 98/41861 is concerned
25 with assaying for the presence of a low molecular
26 weight isoform of cytochrome b5. Cytochrome b5 is
27 involved with cytochrome P450c17 in the synthesis of
28 androstenone. Although data relating levels of
29 cytochrome b5 to levels of androstenone are presented

1 no evidence of a genetic component of the differences
2 is presented.

3

4 Neither the methods of WO 99/18192 nor WO 98/41861
5 address the contribution of skatole or indole.

6 Skatole is critical to consideration of 'boar taint'.

7 While about 25% of consumers are not able to smell
8 androstenone (Claus, 1978. Wien. Tierartztzl Mschr
9 65, 381) skatole is detected by all consumers.

10 Moreover, as skatole formation is not limited to the
11 boar, an understanding of skatole production and
12 clearance may be valuable in other meat species.

13

14 Previous research has suggested that part of the
15 variation in boar taint or its component traits is
16 under genetic control.

17

18 Willeke et al., (Willeke et al., 1987. Selection for
19 high and low level of 5-androst-16-en-3-one in boars.

20 I. Direct and correlated response of endocrinological
21 traits. Journal of Animal Breeding and Genetics 104,
22 64-73) and Sellier and Bonneau (Sellier and Bonneau,

23 1988. Genetic relationships between fat androstenone

24 level in males and development of male and female

25 genital tracts in pigs. Journal of Animal Breeding

26 and Genetics 105, 11-20) have shown that selection

27 (i.e. selective breeding) on fat androstenone level

28 in boars can be effective. Keller et al. (Keller et

29 al., 1997. Influencing the androstenone

30 concentration of entire male pigs by mating AI boars

1 with known fat androstenone level. EAAP Working Group
2 "Production and utilisation of meat from entire male
3 pigs", Stockholm, Sweden, 1-3 October 1997) confirmed
4 that there is a genetic component to androstenone
5 levels. Lundström and co-workers concluded from a
6 study of skatole levels in pig selection lines that
7 there is a genetic effect on skatole deposition which
8 may be due to a recessive allele of a major gene
9 (Lundström et al., 1994. Skatole levels in pigs
10 selected for high lean tissue growth rate on
11 different dietary protein levels. Livestock
12 Production Science 38, 125-132). Fouilloux and
13 colleagues (Fouilloux et al., 1997. Support for
14 single major genes influencing fat androstenone level
15 and development of bulbo-urethral glands in young
16 boars. Genetic Selection Evolution 29, 357-366; Le
17 Roy et al., 1997. Evidence for single major genes
18 influencing fat androstenone level and development of
19 bulbo-urethral glands in young boars. EAAP Working
20 Group "Production and utilisation of meat from entire
21 male pigs", Stockholm, Sweden, 1-3 October 1997)
22 concluded from their data that there is a single
23 major gene influencing androstenone levels in fat.
24 In their model the allele for 'low androstenone
25 levels' is dominant with respect to the allele for
26 'high androstenone levels'. They found no evidence
27 for linkage between the major genes for androstenone
28 levels and bulbo-urethral gland development and the
29 swine leukocyte antigen loci (SLA). However, Bidanel
30 et al. (Bidanel et al., 1997. Chromosome 7 mapping

1 of a quantitative trait locus for fat androstenone
2 level in Meishan x Large White F2 entire male pigs.
3 EAAP Working Group "Production and utilisation of
4 meat from entire male pigs", Stockholm, Sweden, 1-3
5 October 1997) found evidence for an effect on
6 androstenone levels of a gene or genes on chromosome
7 7, close to the *SLA* locus. The androstenone QTL
8 described by Bidanel and colleagues maps to the
9 interval *SLA-S0102* that approximately corresponds to
10 the *TNFB-S0066* interval in our study.

11

12 Genetic selection

13

14 Selection against animals with a genetic
15 predisposition to boar taint would be an attractive,
16 cost-effective and humane solution to the problem of
17 boar taint. The identification of animals of the
18 desired genotype (genetic make up) requires some
19 understanding of the nature of genetic variation and
20 methods to detect it.

21

22 The genome and genetic variation

23

24 The genome of the pig consists of a set of 18 pairs
25 of autosomes and the sex (X and Y) chromosomes found
26 in most cells of the animal. Into these chromosomes
27 is packed a DNA sequence of around 3 billion base
28 pairs in length. This DNA sequence codes for the
29 50,000 to 100,000 genes that control the development
30 of the pig and its appearance, performance and other

1 characteristics. Slight variations in the DNA
2 sequence between animals contribute to differences
3 between animals within breeds and between breeds.
4 The two copies of a gene carried by an animal on
5 alternative members of a homologous chromosome pair
6 may differ from each other in their exact DNA
7 sequence. These alternative variants (or alleles)
8 may or may not encode functionally different
9 products, depending upon the exact nature of the
10 change at the DNA level. Such variation found in a
11 population is referred to as polymorphism and genes
12 or loci displaying variation are said to be
13 polymorphic.

14 An animal's phenotype is the result of complex
15 actions of the genes inherited from its parents and
16 environmental factors. Most traits of agricultural
17 importance in pigs are influenced by variation at
18 several or many different genes. Usually individual
19 genes do not have a large enough effect on their own
20 to produce observable qualitative differences between
21 individuals. More commonly, variation in several or
22 many genes combines to produce continuous or
23 quantitative variation between animals in traits such
24 as growth rate, fatness and predisposition to boar
25 taint.

26

27 Genome mapping can be used to identify the location
28 of genes that influence variation in quantitative
29 traits. For example, if it can be demonstrated that
30 there are significant associations between the

10

1 inheritance of a particular chromosomal region (or
2 locus) and trait variation, that region must contain
3 a gene or genes affecting the trait in question. The
4 loci affecting quantitative traits are termed
5 quantitative trait loci or QTLs.

6
7 The tools used to follow the inheritance in different
8 chromosomal regions are genetic markers and these can
9 be selected from the genome map to ensure coverage of
10 the entire genome. Markers on the genetic map are
11 used to identify a particular region of the genome
12 and follow its inheritance and thus provide the tools
13 to find genes affecting traits of interest.

14
15 The most commonly used markers are microsatellites,
16 where the core of the marker is a tandemly-repeated
17 sequence of two (usually) or a small number of
18 nucleotides, where different alleles are
19 distinguished by having different numbers of repeats.
20 For microsatellites (and for many of the other
21 possible marker types), the polymerase chain reaction
22 (PCR) is used to amplify a small DNA sample and
23 provides the first step in identifying alternative
24 alleles (i.e. genotyping). Unique PCR primers are
25 used to ensure that only alleles of the specific
26 marker of interest are amplified from the DNA sample
27 of an individual animal. The PCR products are then
28 separated by electrophoresis and can be visualised,
29 for example via use of radioactive or fluorescent
30 labels. The use of PCR on DNA-based markers means

11

1 that genotyping can be performed on very small
2 samples of DNA, which can be relatively easily
3 collected at any time. Hence animals can be
4 genotyped as soon as they are born using DNA isolated
5 from blood, ear notches or other material.

6
7 The genetic map can be built in a number of ways,
8 however, the principle method is by linkage analysis.
9 If two markers are close together on a chromosome,
10 then the two alleles that are on the same gamete of
11 an individual will tend to be inherited together.
12 The closer together these two loci are, the more
13 likely it is that they will not be separated by
14 recombination and so will appear linked. Alleles at
15 two loci far apart on the same chromosome or on
16 different chromosomes will be inherited independently
17 and so will produce a proportion of 0.5 recombinant
18 and 0.5 non-recombinant gametes. Hence the frequency
19 of recombinants (the recombination fraction) provides
20 a measure of the distance between two loci. Maps
21 showing distances between ordered loci can be built
22 using recombination frequencies between pairs of loci
23 or between multiple groups of loci.

24
25 Linkage maps of the porcine genome now contain
26 substantial amounts of information and their status
27 is constantly changing. Published linkage maps and
28 linkage data are stored in the pig genome database
29 (PiGBASE / ARKdb-pig: URL =

1 [http://www.ri.bbsrc.ac.uk/pigmap/pig_genome_mapping.h](http://www.ri.bbsrc.ac.uk/pigmap/pig_genome_mapping.html)
2 [tml](http://www.ri.bbsrc.ac.uk/pigmap/pig_genome_mapping.html).

3

4 The basic principle of showing that a gene or a
5 region of the genome is associated with variation is
6 illustrated in Figure 11. It consists of identifying
7 a genetic marker and showing that its inheritance in
8 a suitable pedigree is associated with variation in
9 performance.

10

11 In a population such as that derived from the cross
12 between two lines illustrated in Figure 11, there may
13 be an overall association between a particular marker
14 allele and a particular allele at a quantitative
15 trait locus (QTL). In other words, on average,
16 across all individuals no matter which family they
17 come from, there is a tendency for a particular
18 marker allele to be associated with a particular QTL
19 allele. Such an association is often referred to as
20 linkage disequilibrium. Linkage disequilibrium
21 between a QTL and a marker leads to an overall
22 association between the marker allele and the
23 quantitative trait. In a random mating population,
24 recombination will lead to the gradual decay in
25 linkage disequilibrium between loci, with the rate of
26 decay related to the distance between the loci.

27

28 In the analysis of data, one can look for an overall
29 association between a marker and a quantitative trait
30 (an association study). In such an analysis one is

13

1 making the assumption that the marker and the QTL are
2 in linkage disequilibrium, perhaps because they are
3 very close together (e.g. within the same candidate
4 gene), or because the population is not long
5 established. However, even if a marker and a QTL are
6 very close together, there is no guarantee that
7 linkage disequilibrium between them exists (except in
8 special circumstances, such as a cross between inbred
9 lines) and so a QTL may be missed if association
10 analysis is performed alone. Linkage analysis is a
11 more robust test, as it will detect both associations
12 that vary between families and those that are
13 consistent across the population. However, depending
14 on the population structure, it may be more difficult
15 to perform linkage analysis than association
16 analysis. This is particularly because linkage
17 analysis requires the data to be sampled in a
18 designed manner from a population carefully
19 structured into families, whereas association
20 analysis can be performed on a random sample of
21 individuals. Thus linkage analysis is not always
22 carried out, even though it would be optimum to
23 perform both types of analysis.

24
25 Genome studies often analyse several or many
26 different markers when looking for an effect on the
27 phenotype. Thus, a number of effects may be
28 significant by chance if the standard 5% significance
29 level is used. Hence, it is recommended practise to
30 use a more stringent significance level such that the

1 overall chance of finding a significant result
2 amongst all the markers tested is no more than 5%
3 (see Lander and Kruglyak, 1995, for a more detailed
4 discussion of these points). This means that
5 significance levels as high as 0.01-0.001% may be
6 used in some studies. This in turn increases the
7 sample size required for results to be significant at
8 this level. The samples sizes required to be
9 confident of detecting an effect depend on factors
10 such as the magnitude of the influence on the trait,
11 the type of population studied and the exact analysis
12 to be performed. However, even in the most
13 straightforward situation and with the most carefully
14 designed studies, the minimum sample sizes are likely
15 to be two hundred animals or more.

16
17 The full power of the map and markers is employed in
18 performing a genome scan for loci affecting traits of
19 interest. The strength of this approach is that it
20 has the potential to detect any loci with a large
21 effect on a studied trait, whether or not their
22 existence is known in advance. To implement this
23 approach markers which are spaced at intervals
24 through the genome and which are polymorphic in the
25 population being studied are selected from the map.
26 The phenomenon of genetic linkage means that each
27 marker can be used to follow the inheritance of a
28 section of linked chromosome. Around 100-150 evenly
29 spaced markers are needed to cover the whole genome
30 and follow the inheritance of all sections. Thus

1 maps of highly polymorphic markers are very valuable
2 for this approach, as they allow selection of markers
3 that provide this coverage and that are informative
4 in the population of interest.

5

6 Thus the genome scan can both localise known genes of
7 major effect and identify loci that were not known a
8 *priori*. A significant amount of work is required to
9 type sufficient animals for markers covering the
10 entire genome. However, it is possible to design an
11 experiment such that there is a high probability of
12 detecting a gene of a defined effect on the phenotype
13 wherever it is in the genome.

14

15 We have conducted such a genome scan for QTL
16 contributing to variation in boar taint and its
17 component traits.

18

19 We have identified QTL for boar taint and its
20 component traits. Of most interest are QTL for boar
21 taint traits located on chromosome 6 (in a region
22 defined by the markers SW782, SW1057, S0121 and
23 SW322) and on chromosome 14 (in a region defined by
24 the markers SW857, SW2496, SW295, SW210, S0007, SW761
25 and SW1557). We have also identified further QTL
26 with smaller effects for different components of boar
27 taint on several other chromosomes (e.g. 1, 2, 3, 4,
28 5, 8, 9, 10, 11, 13, 18 and X).

29

1 Thus, in one aspect, the present invention provides
2 genetic markers for characteristics of boar taint,
3 derived from:

- 4
- 5 i) SW782, SW1057, S0121, SW322 or regions of
6 chromosome 6 spanning therebetween (preferably
7 between positions 40 to 120 of chromosome 6); or
 - 8 ii) SW857, SW2496, SW295, SW210, S0007, SW761,
9 SW1557, SW2515, SWC27 or regions of chromosome
10 14 spanning therebetween (preferably between
11 positions 10 to 70 of chromosome 14).
- 12

13 The specific markers referred to above detailed in
14 the website

15 <http://www.ri.bbsrc.ac.uk/pigmap/pigbase/pigbase.html>

16 and specifically can be accessed via

17 <http://www.ri.bbsrc.ac.uk/pigmap/pigbase/loclist.html>

18

19 Brief details of these markers are also set out in
20 the example.

21

22 In a further aspect, the present invention provides
23 an assay to identify pigs with a genetic
24 predisposition that reduces the incidence of boar
25 taint, wherein said assay comprises:

- 26 a) obtaining a DNA sample from a test pig;
- 27 b) analysing the sample to determine the allelic
28 variant(s) present at a genetic marker, wherein
29 said markers are selected from:

17

- 1 i) SW782, SW1057, S0121, SW322, or regions of
2 chromosome 6 spanning therebetween
3 (preferably between positions 40 to 120 of
4 chromosome 6); or
5 ii) SW857, SW2496, SW295, SW210, S0007, SW761,
6 SW1557, SW2515, SWC27 or regions of
7 chromosome 14 spanning therebetween
8 (preferably between positions 10 to 70 of
9 chromosome 14; and
10 c) using said marker results to select for animals
11 of the preferred genotype.

12
13 In a yet further aspect, the present invention
14 provides a method of identifying boars which have a
15 genetic disposition to reduced boar taint, said
16 method comprising:

- 17
18 a) obtaining a DNA sample from said boar;
19 b) assaying said boar for a sequence identical or
20 complementary to the genetic markers identified
21 above.

22
23 Although the study looked at the particular markers
24 identified above, it is known to those skilled in the
25 art that other genetic markers from within the QTL or
26 the neighbouring portions of chromosome 6 or 14 (as
27 appropriate) may be used instead, provided of course
28 that the marker(s) selected are found to map within
29 or close to the QTL for boar taint traits.

1 Thus, the present invention provides a method to
2 identify pigs with a genetic predisposition that
3 reduces the incidence of boar taint, wherein said
4 method comprises:
5 a) obtaining DNA samples from a population of pigs;
6 b) genotyping at least a sample of said population
7 for pre-determined markers that map within or
8 close to the QTL for boar taint traits defined
9 herein (preferably on chromosomes 6 and 14, for
10 example the specific markers referred to above
11 or other markers located on either of
12 chromosomes 6 and 14 where a high F ratio is
13 indicated in any of Figs. 1 to 10);
14 c) measuring boar taint traits for at least a
15 sample of said population;
16 d) correlating the presence of allelic variants of
17 said markers with said traits;
18 e) obtaining a DNA sample from a test pig;
19 f) analysing the sample to determine the allelic
20 variant(s) present at a said genetic marker; and
21 g) using said marker results to select for animals
22 of the preferred genotype.

23

24 Steps a) and d) of the method described above are
25 concerned with identifying markers which map within
26 or close to the QTL for boar taint traits or with
27 confirmation that the particular markers referred to
28 are also relevant for the test population.

29 Preferably the markers are derived from SW782,
30 SW1057, S0121, SW322, SW857, SW295, S0007 or SW1557.

1 Other markers that map within or close to the QTL
2 described herein can also be used. Particular
3 mention may be made of any marker located within
4 positions 40 to 120 of chromosome 6, or within
5 positions 10 to 70 of chromosome 14. As can be seen
6 in Figs. 1 to 10 certain areas of chromosomes 6 to 14
7 correlate to high F ratios for specific traits
8 connected to boar taint and markers in these regions
9 may be of particular interest.

10

11 Optionally, a selection of markers that each allow
12 the allelic variation at different QTL associated
13 with boar taint to be predicted may be used in
14 combination to achieve a more accurate prediction of
15 boar taint predisposition. The present invention
16 thus provides a kit comprising at least two such
17 markers, preferably selected from the specific
18 markers recited above.

19

20 The animals shown to have marker genotypes or
21 predicted QTL genotypes indicative of a desirable
22 boar taint predisposition (for example boars
23 identified to have reduced boar taint), or the close
24 relatives of such animals, can be used as breeding
25 stock or for meat production.

26

27 Although the genetic markers used in this study are
28 microsatellites the assay is not limited to the use
29 of any particular technology or type of genetic
30 marker. Any method for detecting DNA variation at

1 specific chromosomal locations can be used to develop
2 genetic markers that could be used for monitoring the
3 inheritance of particular chromosomal segments or
4 loci. It is clear to those skilled in the art that
5 genetic markers, which map close to or within the QTL
6 for boar taint traits defined herein, could be used
7 in the assay for predicting an individual's
8 predisposition to boar taint traits independent of
9 the technology used to develop or genotype the
10 marker. Thus, the assay is not limited to any
11 particular type of genetic marker or genotyping
12 technology, current or as yet undeveloped. Other
13 genetic marker types and technologies include, but
14 are not limited to, restriction fragment length
15 polymorphisms (RFLPs), single strand conformational
16 polymorphisms (SSCP), double strand conformational
17 polymorphisms, single nucleotide polymorphisms
18 (SNPs), AFLP™ (amplified fragment length
19 polymorphisms, DNA chips, variable number of tandem
20 repeats (VNTRs, minisatellites), random amplified
21 polymorphic DNA (RAPDs), heteroduplex analyses, and
22 allele-specific oligonucleotides (ASOs). Some DNA
23 variation can be detected by assaying the variation
24 in RNA transcripts or proteins. Thus, genetic marker
25 technology for the purposes of the assay is not
26 limited to direct measures of DNA variation.
27 Examples of markers that map to the boar taint QTL on
28 chromosome 6 and 14 include, but are not limited to,
29 (marker type and chromosome are shown in parenthesis)
30 UBC (RFLP, SSC14); ACTA1 (PCR-RFLP, SSC14); S0063

1 (microsatellite, SSC14); GPI (RFLP, VNTR, protein
2 variants, SSC6); PGD (SSCP, protein variants); TTR
3 (SSCP, PCR-RFLP, SSC6); S0299 (microsatellite, SSC6).

4 Details of genetic marker technology can be accessed
5 in primary research publications, review articles,
6 textbooks and laboratory manuals.

7 In the assay of the present invention, the genomic
8 DNA will be detected from a sample of porcine origin
9 but the exact tissue forming the sample is not
10 critical as long as it contains genomic DNA.

11 Examples include body fluids such as blood, sperm,
12 ascites and urine; tissue cells such as liver tissue,
13 muscle, skin, hair follicles, fat and testicular
14 tissue. The genomic DNA to be analysed can be
15 prepared by extracting and purifying the DNA from
16 such samples.

17

18 The method may be conducted *in vitro* or *in vivo* using
19 a sample from a living animal or post mortem
20 following the death of the animal being tested. If
21 the assay is conducted post mortem, the information
22 obtained may be of use for the siblings, parents or
23 other close relatives of the animal.

24

25 The QTL for boar taint traits disclosed herein will
26 allow the isolation and characterisation of the
27 trait-genes themselves, since the positioning of the
28 QTL enables a search for linkage to the genes
29 responsible for the trait. Once these trait genes
30 are located the option to manipulate the trait genes

1 by transgenesis or to develop a further assay arises
2 and forms part of the present invention.

3

4 The present invention will now be described in more
5 detail by reference to the following, non-limiting,
6 example and figures in which:

7

8 Figure 1 and Figures 3 to 6 are graphs plotting the F
9 value against position (cM) on chromosome 6 for
10 different boar taint related traits.

11

12 Figure 2 and Figures 7 to 10 are graphs plotting the
13 F value against position (cM) on chromosome 14 for
14 different boar taint related traits.

15

16 Figure 11 depicts a three-generation pig pedigree
17 produced by crossing divergent purebred lines of pigs
18 to produce F₁ and F₂ generations. We focus on one
19 small part of a single chromosome that carries a
20 genetic marker with alternative alleles 1 and 2. The
21 animals can be genotyped for this marker and the
22 inheritance of alternative alleles can be followed
23 through the pedigree. In the F₂ animals, both the
24 marker and genes controlling the size differences
25 between the breeds segregate. The marker acts as a
26 signpost to show from which breed linked sections of
27 chromosome are inherited. In this example the size
28 of F₂ animals is associated with the marker genotype
29 (animals with the 11 genotype are large, those with
30 22 are small). Hence a gene or genes for size is

1 found in the region of chromosome inherited with the
2 marker.

3
4 Figures 12 to 15 show graphs plotting the F value
5 against position (cM) on chromosome 14 for boar taint
6 related traits established through an alternative
7 analysis.

8
9 Figure 16 shows a graph depicting the association of
10 within sire QTL estimates for laboratory taint
11 measures with those assessed by the taste panel.

12
13 **Example 1**

14
15 QTL mapping pedigrees were established in the form of
16 three-generation families in which grandparents from
17 genetically divergent breeds were crossed to produce
18 the parental (F₁) generation which were subsequently
19 intercrossed. The founder grandparental breeds were
20 the Chinese Meishan and the European Large White
21 (Yorkshire). 308 F₂ animals were produced in these
22 Large White/Meishan pedigrees on the Roslin
23 Institute's farm at Mountmarle, Midlothian, Scotland.

24
25 Blood samples were taken by venepuncture from most
26 grandparental, F₁ parental and F₂ pigs. DNA was
27 prepared from blood samples.

28
29 In the early part of the trial animals were penned in
30 like-sex groups of 4 and fed *ad libitum* during the

1 growing period. Hunday electronic feeders and weight
2 crates were introduced for half of the second batch
3 and all of the third batch of animals. Animals were
4 penned in groups of 12-13 and fed *ad libitum* using
5 this equipment. A comparison in the second batch
6 showed no major differences in growth between animals
7 penned in groups of 4 and those in larger groups with
8 electronic feeders.

9

10 The animals were transported to the University of
11 Bristol for slaughter at around 85 kg in weight.
12 Phenotypic markers or component traits indicative of
13 boar taint were analysed.

14

15 Tissue samples were taken from all F₂ animals and
16 stored at -70°C as a source for the preparation of
17 DNA. DNA was prepared from frozen tissue (spleen)
18 samples.

19

20 The phenotype markers were:

- 21 i) taste panel assessment of abnormal odour;
- 22 ii) taste panel assessment of boar flavour in lean
23 meat;
- 24 iii) taste panel assessment of abnormal flavour in
25 lean meat;
- 26 iv) taste panel assessment of boar flavour in fat;
- 27 v) taste panel assessment of abnormal flavour in
28 fat;
- 29 vi) taste panel assessment of skatole;
- 30 vii) taste panel assessment of androstenone;

1 viii) taste panel assessment of overall
2 acceptability.

3 ix) laboratory measure of indole;

4 x) laboratory measure of skatole;

5 xi) laboratory measure of androstenone;

6
7 Analysis of the phenotype markers at the University of
8 Bristol was conducted by taste panels for items ix, x and
9 xi using chemical analysis as described by Annor-Frempong
10 et al., Meat Science 47:49-61, 1997; and de Brabander et
11 al., "Boar Taint in Belgian pigs in relation to the
12 androstenone content", Proc. 31st Europ. Meet. Res. Works,
13 Vama, 778-781, 1985. The remaining phenotype markers (i-
14 viii) were measured by the trained taste panel at the Meat
15 and Livestock Commission. Two samples of meat for each
16 animal were assessed in separate sessions by a trained
17 sensory panel. Over the three years of data collection,
18 there was a total of 117 sessions, and 59 panellists were
19 involved at some stage of the procedure, with 22
20 panellists appearing in all three years. At each panel
21 session, meat samples from six animals were weighed raw,
22 cooked, then weighed again to determine cooking loss. Each
23 of five to seven panellists at that session was then given
24 a separate sample of lean and fat from each of the six
25 animals. Each panellist gave each animal a score for each
26 of thirteen attributes, on a scale of 1-24 (the higher the
27 better) by marking a prepared form. The lean sample was
28 assessed by mouth for juiciness, tenderness, pork flavour,
29 abnormal flavour and boar flavour. The fat sample was
30 assessed by mouth for pork flavour, abnormal flavour and

26

1 boar flavour and by nose for pork odour, abnormal odour,
2 androstenone and skatole. Finally, a score was given for
3 overall acceptability.

4

5 Each session and panellist involved in the trial had a
6 unique number. The scores awarded by the panellists were
7 analysed using the restricted maximum likelihood in a
8 model fitting session number, panellist and individual
9 animal number. Fitted values for each attribute for each
10 individual were saved from these analyses and stored on a
11 database for use in the QTL analyses.

12

13 DNA and tissue samples were shipped to Perkin-Elmer
14 Agen (PE-Agen) for genotyping. Genotyping was
15 performed using fluorescently labelled primers on ABI
16 semi-automated DNA sequencers. The size of the
17 labelled PCR products as resolved on ABI semi-
18 automated DNA sequencers was estimated using ABI
19 proprietary software (Genescan™ and Genotyper™).
20 Genotyping results were returned to the Roslin
21 Institute on CD-ROM. The results were loaded into
22 the project database (resSpecies-pig
23 <http://www.ri.bbsrc.ac.uk/bioinformatics/databases>).

24

25 Details of the pedigree structure, dates of birth,
26 sex and growth and feed intake were loaded into
27 resSpecies from the farm database.

28

1 The collated data on traits and marker genotypes were
 2 analysed to scan the genome for the presence of QTL
 3 influencing the traits of interest.

4

5 The animals were genotyped for the genetic markers
 6 listed in Table 1. The markers were chosen to
 7 provide a reasonable spread over the whole of the
 8 genome.

9

10 Table 1: Markers used for genome scan.

Marker	Chromosome	Position (cM)
SW1515	1	0.0
CGA	1	41.9
S0082	1	69.8
S0155	1	77.3
SW1828	1	105.7
SW373	1	109.1
SW1301	1	131.2
SW2443	2	0.0
SW256	2	20.1
SW240	2	49.2
S0226	2	73.7
S0378	2	92.3
S0036	2	130.9
SW72	3	0.0
SW2527	3	20.8
SW902	3	39.2
S0167	3	70.1
S0002	3	92.0
SW590	3	116.3
S0227	4	0.0
S0301	4	23.9

28

Marker	Chromosome	Position (cM)
S0001	4	43.4
S0217	4	61.5
S0073	4	67.8
SW445	4	99.4
S0097	4	117.1
DAGK	5	0.0
S0005	5	15.2
IGF1	5	40.8
SW1954	5	54.2
SW967	5	77.3
SW2535	6	0.0
SW1057	6	38.1
SW782	6	72.5
S0121	6	101.5
SW322	6	132.6
SW2419	6	144.4
S0025	7	0.0
SW2155	7	34.9
TNFB	7	59.6
S0066	7	76.8
SW632	7	98.7
S0101	7	124.0
SW764	7	145.4
SW2611	8	0.0
S0017	8	72.0
S0225	8	87.6
SW61	8	111.2
S0178	8	144.9
SW983	9	0.0
SW911	9	34.7
SW1677	9	69.3
SW2093	9	92.0
SW1651	9	166.0

Marker	Chromosome	Position (cM)
SW830	10	0.0
SW443	10	31.7
SW497	10	54.0
SW1041	10	70.3
SW951	10	98.8
SWR67	10	129.9
S0385	11	0.0
SW1632	11	18.8
S0071	11	41.2
S0230	11	51.6
SW703	11	70.0
S0143	12	0.0
SW957	12	19.3
S0090	12	49.9
SW1378	13	0.0
S0076	13	14.9
S0068	13	53.3
SW398	13	71.7
SW1056	13	93.3
S0215	13	113.3
SW857	14	0.0
SW2496	14	15.1
SW295	14	41.5
S0007	14	53.2
SW761	14	70.6
SW1557	14	83.0
SW2515	14	103.8
SWC27	14	110.9
S0355	15	0.0
S0148	15	14.5
SW964	15	26.7
SW936	15	54.3
SW1119	15	84.4

30

Marker	Chromosome	Position (cM)
S0111	16	0.0
S0006	16	51.5
S0026	16	89.5
SW1897	16	110.0
SW24	17	0.0
SW1920	17	31.1
S0332	17	63.4
SW2540	18	0.0
SW1984	18	28.8
SW1682	18	41.0
SW949	X	0.0
SW2534	X	57.8
SW2456	X	70.1
SW1943	X	82.5
S0218	X	94.2

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Linkage maps of each pig chromosome were developed using Cri-Map version 2.4 (Green, P., Falls, K. and Crooks, S. (1990), Documentation for Cri-Map version 2.4. St. Louis, Washington University School of Medicine). The linkage map positions for the markers on chromosomes 6 and 14 are presented in Table 1.

The trait data and linkage maps were analysed by the least squares approach as described by Haley et al., Genetics, 136:1195-1207, 1994. Due to the non-normality of the laboratory measured traits indole, skatole and androstenone, data for these traits were log-transformed prior to analysis. All chromosomes were tested in this way (using appropriate markers for the chromosome under test), but the most

31

1 significant correlation was found for boar taint in
2 the markers for chromosomes 6 and 14.

3

4 Other more minor effects for the laboratory measured
5 traits are given below in Table 2 (two sexes analysed
6 separately and with log transformed data):

7

8 Table 2

9

Chromosome	Trait
2	Skatole
4	Skatole, androstenone
7	Androsténone
8	Androstenone, indole
9	Androstenone
11	Skatole, androstenone, indole
12	Skatole
13	Androstenone, indole
16	Androstenone
17	Androstenone
X	Skatole, androstenone, indole

10

11

12 Brief details of the markers found to map to QTL for
13 boar taint are given below:

14

15 SW782: Rohrer et al., "A microsatellite linkage
16 map of the porcine genome", Genetics 136:231-45,
17 1994.

18

32

1 **Method: Microsatellite**
2 **Forward Primer:** TCTTCACATATGAGCACCAACC
3 **Reverse Primer:** CGGAACAAGAGGAAGTGAGTG
4 **PCR Conditions:**
5 **Anneal temp** 60.000°C
6 **Mg²⁺conc** 1.500 mM
7 **dNTPs-conc** 30.00 µM
8 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
9 primer, 0.45 units Taq polymerase. For further
10 details of allele size range and heterozygosity see
11 <http://sol.marc.usda.gov>.
12 **Gel Details:**
13 **Matrix:** polyacrylamide **Concentration:** 7.000 g/100ml
14 S0121 (6 q3.1-q3.5): Robic et al., "Porcine
15 linkage and cytogenetic maps integrated by regional
16 mapping of 100 microsatellites on somatic cell hybrid
17 panel", Mammalian Genome 7:438:445, 1996.
18
19 EMBL Accession No L30152
20
21 **Method: Microsatellite**
22 **Forward Primer:** TTGTACAATCCCAGTGGAATCC
23 **Reverse Primer:** AATAGGGCATGAGGGTGTTTGA
24 **PCR Conditions:**
25 **Anneal temp** 55.000°C
26 **Mg²⁺conc** 2.000 mM
27 **dNTPs-conc** 200.000 µM

33

1 **Cycle profile** 6 min at 94°C, 30 x 1 min at 55°C; 1
2 min at 72°C; 1 min at 94°C, followed by a final
3 extension of 7 min at 72°C.

4 **Gel Details:**

5 **Matrix** polyacrylamide

6 **Concentration** 6.000 g/100ml

7 **Additives** 7M urea

8

9 SW322 (6 q3.1-q3.5): Rohrer et al., 1994, supra;
10 Robic et al., 1996, supra.

11

12 **Method: Microsatellite**

13 **Forward Primer:** CATTCAACCTGGAATCTGGG

14 **Reverse Primer:** TCCCTGGAAAGGCTACACC

15

16 **PCR Conditions**

17 **Anneal temp** 62.000°C

18 **Mg++conc** 1.500mM

19 **dNTPs-conc** 30.000µM

20 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
21 primer, 0.45 units Taq polymerase. For further
22 details of allele size range and heterozygosity see
23 <http://sol.marc.usda.gov>

24 **Gel Details**

25 **Matrix:** polyacrylamide

26 **Concentration** 7.000 g/100ml

27

34

1 SW857 (14 q2.1-q2.2): Lopez-Corrales et al.,
2 "Cytogenic assignment of 53 microsatellites from the
3 USDA-MARC porcine genetic map", Cytogenetics and Cell
4 Genetics 84:140-144, 1999.

5

6 **Method: Microsatellite**

7 **Forward Primer:** TGAGAGGTCAGTTACAGAAGACC

8 **Reverse Primer:** GATCCTCCTCCAAATCCCAT

9 **PCR Conditions:**

10 **Anneal temp** 58.000°C

11 **Mg²⁺conc** 1.500 mM

12 **dNTPs-conc** 30.000 µM

13 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
14 primer, 0.45 units Taq polymerase. For further
15 details of allele size range and heterozygosity see
16 <http://sol.marc.usda.gov>.

17 **Gel Details:**

18 **Matrix** polyacrylamide

19 **Concentration** 7.000 g/100ml

20

21 SW295 (14 q2.2-q2.4): Robic et al., 1996, supra.

22 **Method: Microsatellite**

23 **Forward Primer:** ACCTGCCAGAGTTGTGGC

24 **Reverse Primer:** AAGAGTTTCATTTCTCCCATCC

25 **PCR Conditions:**

26 **Anneal temp** 62.000°C

27 **Mg²⁺conc** 1.500 mM

28 **dNTPs-conc** 30.000 µM

29 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
30 primer, 0.45 units Taq polymerase. For further

35

1 details of allele size range and heterozygosity see
2 <http://sol.marc.usda.gov>.

3 **Gel Details:**

4 **Matrix** polyacrylamide

5 **Concentration** 7.000 g/100ml

6

7 S0007 (14) Fredholm et al., "Characterization of
8 24 porcine (dA-dC)n-(dT-dG)n microsatellites:
9 genotyping of unrelated animals from four breeds and
10 linkage studies", Mammalian Genome 4:187-92, 1993.

11

12 EMBL Accession No M97234

13

14 **Method: Microsatellite**

15 **Forward Primer:** TTACTTCTTTGGATCATGTC

16 **Reverse Primer:** GTCCCTCCTCATAATTTCTG

17 **PCR Conditions:**

18 **Anneal temp** 56.000°C

19 **Mg²⁺conc** 1.500 mM

20 **Salt-conc** 50.000 mM

21 **dNTPs-conc** 200.000 µM

22 **Cycle profile** 1 x 94°C, 3 min; 56°C, 1 min; 72°C, 30
23 sec; then 30 x 94°C, 30 sec; 56°C, 1 min; 72°C, 5 min.

24 **PCR-Annotation** Hybaid thermal cycler

25 **Gel Details:**

26 **Matrix** polyacrylamide

27 **Concentration** 6.000 g/100ml

28 **Additives** denaturing gel

29

36

1 SW1557 (14) Alexander et al., "Cloning and
2 characterization of 414 polymorphic porcine
3 microsatellites", Animal Genetics 27:137-148, 1996.

4 **Method: Microsatellite**

5 **Forward Primer:** TGCTCTAATCTACCCGGGTC

6 **Reverse Primer:** CCACCCCACTCCCTTCTG

7 **PCR Conditions:**

8 **Anneal temp** 58.000°C

9 **Mg²⁺conc** 1.500 mM

10 **dNTPs-conc** 30.000 µM

11 **Cycle profile** 92°C, 2 min; 30 x 94°C, 30 sec, anneal
12 temp 30 sec, 72°C 30 sec; 1 x 72°C, 5 min.

13 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
14 primer, 0.45 units Taq polymerase. For further
15 details of allele size range and heterozygosity see
16 USDA-MARC database - <http://sol.marc.usda.gov>.

17 **Gel Details:**

18 **Matrix** polyacrylamide

19 **Concentration** 7.000 g/100ml

20

21 SW2496 (14 q2.1-q2.2): Lopez-Corrales et al.
22 "Cytogenetic assignment of 53 microsatellites from
23 the USDA-MARC porcine genetic map", Cytogenetics and
24 Cell Genetics 84:140-144, 1999.

25

26 **Method: Microsatellite**

27 **Forward Primer:** TGAGAGGTCAGTTACAGAAGACC

28 **Reverse Primer:** GATCCTCCTCCAAATCCCAT

37

1 **PCR Conditions**2 **Anneal temp** 58.000°C3 **Mg++conc** 1.500mM4 **dNTPs-conc** 30.000µM

5 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
6 primer, 0.45 units Taq polymerase. For further
7 details of allele size range and heterozygosity see
8 <http://sol.marc.usda.gov>

9 **Gel Details**10 **Matrix:** polyacrylamide11 **Concentration** 7.000 g/100ml

12

13 SW210: Rohrer et al. "A microsatellite linkage map
14 of the porcine genome." Genetics 136:231-45, 1994.

15

16 **Method: Microsatellite**17 **Forward Primer:** TCATCACCATCATACCAAGATG18 **Reverse Primer:** AATTCTGCCAAGAAGAGAGCC19 **PCR Conditions**20 **Anneal temp** 60.000°C21 **Mg++conc** 1.500mM22 **dNTPs-conc** 30.000µM

23 **PCR-Annotation:** 12.5 ng DNA template, 5 pmol each
24 primer, 0.45 units Taq polymerase. For further
25 details of allele size range and heterozygosity see
26 <http://sol.marc.usda.gov>

27 **Gel Details**28 **Matrix:** polyacrylamide29 **Concentration** . 7.000 g/100ml

30

1 SW761 Rohrer et al. "A microsatellite linkage map of
2 the porcine genome." Genetics 136:231-45, 1994.

3

4 **Method: Microsatellite**

5 **Forward Primer:** CTTTGCTCCCCATTAAGCTG

6 **Reverse Primer:** TCTAGCAAATGTCTGAGATGCC

7 **PCR Conditions**

8 **Anneal temp** 60.000°C

9 **Mg++conc** 1.500mM

10 **dNTPs-conc** 30.000µM

11 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
12 primer, 0.45 units Taq polymerase. For further
13 details of allele size range and heterozygosity see
14 <http://sol.marc.usda.gov>

15

16 **Gel Details**

17 **Matrix:** polyacrylamide

18 **Concentration** 7.000 g/100ml

19

20 SW2515 (14 q 2.9) Alexander et al. "Physical
21 assignments of 68 porcine cosmids and lambda clones
22 containing microsatellites." Mammalian Genome 7:368-
23 372, 1996.

24

25 **Method: Microsatellite**

26 **Forward Primer:** CCATCTCATCCAGAAACATCC

27 **Reverse Primer:** AGGATGCTGAGGTGTTAGGC

28 **PCR Conditions**

29 **Anneal temp** 60.000°C

30 **Mg++conc** 1.500mM

1 **dNTPs-conc** 30.000 μ M

2 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
3 primer, 0.45 units Taq polymerase. For further
4 details of allele size range and heterozygosity see
5 <http://sol.marc.usda.gov>

6

7 **Gel Details**

8 **Matrix:** polyacrylamide

9 **Concentration** 7.000 g/100ml

10

11 SWC27 (14 q2.8-q2.9) Alexander et al. "Physical
12 assignments of 68 porcine cosmids and lambda clones
13 containing microsatellites." Mammalian Genome 7:368-
14 372, 1996.

15

16 **Method: Microsatellite**

17 **Forward Primer:** CATTCAACCTGGAATCTGGG

18 **Reverse Primer:** TCCCTGGAAAGGCTACACC

19 **PCR Conditions**

20 **Anneal temp** 58.000°C

21 **Mg++conc** 1.500mM

22 **dNTPs-conc** 30.000 μ M

23 **PCR-Annotation** 12.5 ng DNA template, 5 pmol each
24 primer, 0.45 units Taq polymerase. For further
25 deatils of allele size range and heterozygosity see
26 <http://sol.marc.usda.gov>

27

1 **Gel Details**

2 **Matrix:** polyacrylamide

3 **Concentration** 7.000 g/100ml

4

5 **QTL Analyses**

6

7 All QTL analyses were performed by least squares.

8 The assumption underlying these analyses is that QTL
9 of major (i.e. detectable) effects were fixed for
10 alternative alleles in the Meishan and Large White
11 breeds that went into the study.

12

13 Several alternative models were used in the QTL
14 analyses. The basic models included fixed effects
15 and any key covariates. Sex was always included as
16 was either year or slaughter data as a fixed effect.
17 For traits where QTL effects may differ between sexes
18 a model including a QTL x sex interaction (estimating
19 a separate QTL effect for both sexes) was used in
20 addition to the basic model.

21

22 **Results**

23

24 The significant results for log transformed data and
25 analysis allowing for differences between the sexes
26 are set out in Table 3.

27

28 From Table 3 it can be seen that when analysis of
29 androstenone, indole and skatole was performed on the

1 basis of the sex of the animal, it was found that no
2 QTL effect was present in female pigs, as expected
3 (estimates of additive and dominance effects in
4 females were not significantly different from zero),
5 but significant effects were found in males.

6
7 The results of the analysis for chromosome 6 are
8 summarised in Figure 1 for laboratory measurements of
9 taint associated compounds and in Figures 3 to 6 for
10 traits recorded by the taste panel. These Figures
11 show that high F values peak on chromosome 6 at
12 positions 40 to 120.

13
14 The results of the analysis for chromosome 14 are
15 summarised in Figure 2 for laboratory measurements of
16 taint associated compounds and in Figures 7 to 10 for
17 traits recorded by the taste panel. These Figures
18 show that high F values peak on chromosome 14 at
19 positions 10 to 70.

20
21 Unexpectedly, and contra-indicated by the literature,
22 our results indicate an association between skatole
23 and androstenone and this ability to use both markers
24 together to measure boar taint predisposition will
25 significantly enhance the accuracy of the assay.

26

27 **Further QTL Analysis**

28

29 In view of the findings and conclusions drawn from
30 the QTL analysis as set out above, further analysis

1 was carried out, this analysis looking specifically
2 at log transformed laboratory measures of indole and
3 skatole, as well as the most important measures of
4 taint as assessed by the sensory panel.

5
6 It should be noted that these analyses, unlike the
7 analysis previously shown, was carried out using data
8 from males only. The basis for this was that
9 previous analysis had included both sexes, but
10 allowed the QTL effect to differ between sexes.
11 There was however no evidence in the earlier analysis
12 to show any effect of detected QTL in females, hence
13 females are excluded from the present analysis.

14
15 This analysis further served to establish a new trait
16 by summing the laboratory measures of indole and
17 skatole and include a measure of the log (indole +
18 skatole) in the analysis, wherein these measurements
19 were only analysed separately in the previous
20 analysis.

21
22 An additional analysis was included that looked at
23 whether QTL effects differed according to F1 sire
24 (sire interaction). Previous analyses made assumption
25 that any QTL was fixed for alternative alleles in the
26 two breeds (Meishan and Large White) crossed. This
27 means that all F1 parents should be the same for any
28 QTL and all F2 litters should be segregating in a
29 similar manner. This new analysis allows F1 sires to
30 differ from one another, as they would if a QTL was

1 segregating within either or both of the two breeds
2 (Meishan and Large White).

3

4 **Results**

5 Data were available on 180 F2 males, progeny of 11 F1
6 sires.

7

8 Analyses of log transformed data on laboratory
9 measures (skatole, indole and skatole + indole) gave
10 less clear and lower peak at 46 cM (between SW210 and
11 S0007). These peaks were significant at the
12 suggestive level ($F = 6.0$ to 8.3).

13

14 Sensory panel data provided evidence for QTL
15 particularly for 'skatole' ($F = 7.65$ at 31 cM) and
16 fat boar flavour ($F = 5.68$ at 30 cM).

17

18 Detailed estimates from these analyses are shown in
19 table 4.

20

21 Analyses of (log) laboratory indole, skatole and
22 indole+skatole measures including a sire interaction
23 increased the significance level to genome wide
24 significance and the interaction with sire was
25 significant. The estimated QTL position was 51-56 cM,
26 close to S0007. Test statistics and estimated
27 position of the QTL are given in table 5 below.

28

29

30

1 Table 5

Character	Chr.	Position (cM)	F-ratio	F-probability
Boar flavour in lean	14	48	1.54	0.12213
Boar flavour in fat	14	79	2.02	0.02971
Skatole (sensory panel)	14	39	2.56	0.00521
Log skatole (lab)	14	56	3.44	0.00026
Log indole (lab)	14	56	3.91	0.00005
Log indole+skatole (lab)	14	51	4.23	0.00002

2

3 Some sires showed a positive QTL effect and others a
 4 negative QTL effect, although as in the foregoing
 5 analyses, the overall effect was negative (indicating
 6 that an average Large White alleles reduce levels).

7

8 Results were less clear cut for sire interaction
 9 analysis of sensory panel assessment of skatole. To
 10 look at the association of within sire QTL estimates
 11 for laboratory taint measures with those assessed by
 12 the taste panel, estimated the association between
 13 the within sire QTL t-values (estimated within sire
 14 QTL estimate divided by its standard error) for the
 15 two analyses of log (indole+skatole) and the sensory
 16 panel assessment of skatole. The plot of these
 17 estimates for the 11 F1 sires is shown in figure 16.
 18 This figure shows that across sires there are both
 19 negative and positive within sire QTL estimates for
 20 both laboratory and sensory panel taint measures and

45

1 these estimates were well correlated ($r = 0.66$)
2 across sires.

3

4 These results confirm that the QTL must be
5 segregating within one or both of the two breeds
6 originally crossed as well as in the cross between
7 them. The within sire segregation of taint measures
8 recorded in the laboratory provides a good predictor
9 of taint as assessed by a sensory panel. Hence the
10 QTL may potentially be used as a predictor of taint
11 within European populations as well as in
12 experimental crosses.

13

14 **Chromosomal localization of CYP2E candidate gene**

15

16 To localise the (candidate) CYP2E (cytochrome P450,
17 subfamily IIE (ethanol-inducible) gene on the porcine
18 genome, two PCR tests were developed to amplify
19 porcine CYP2E sequences from a porcine - rodent
20 somatic cell hybrid panel of twenty-seven cell lines
21 (Yerle, M., Echard, G., Robic, A., Mairal, A., Dubut
22 Fontana, C., Riquet, J., Pinton, P., Milan, D.,
23 Lahbib-Mansais, Y. and Gellin, J., 1996. A somatic
24 cell hybrid panel for pig regional gene mapping
25 characterized by molecular cytogenetics.
26 Cytogenetics and Cell Genetics 73: 194). The PCR
27 reactions were optimised for temperature, magnesium
28 concentration and the number of cycles to
29 specifically amplify the porcine gene only. One pair
30 of gene-specific oligonucleotide primers (sequences

46

1 CYP2E7.for and CYP2E8.rev) were designed for
2 amplification of a fragment spanning the predicted
3 intron between the predicted exons 7 and 8.

4

5 CYP2E7.for 5'-CATGAGATTCAGCGATTCATCG-3'

6 CYP2E8.rev 5'-TGCTCTGGCTTAAACTTCTCCG-3'

7

8 Both PCR reactions contained the relevant pair of
9 gene-specific oligonucleotide primers at a
10 concentration of 0.2 micromolar and 50 nanograms of
11 porcine / rodent somatic cell hybrid cell line
12 genomic DNA. Control samples included hamster
13 genomic DNA (50 nanograms), mouse genomic DNA (50
14 nanograms) and porcine genomic DNA (50 nanograms).
15 Aliquots of the PCR products were examined by agarose
16 (1.2% w/v) gel electrophoresis. Each gel lane was
17 scored for the presence or absence of the expected
18 porcine-specific CYP2E gene-specific PCR product.
19 Statistical analysis of these data was performed with
20 a computer program available on the World Wide Web
21 (Chevalet, C., Gouzy, J. and SanCristobal Gaudy, M.,
22 1997. Regional assignment of genetic markers using a
23 somatic cell hybrid panel: a WWW interactive program
24 available for the pig genome. Computer Applications
25 in BioScience 13: 69).

26

27 Analysis of the pattern of presence or absence of the
28 pig CYP2E gene-specific sequences across the panel of
29 porcine-rodent somatic cell hybrids suggested that .

47

the CYP2E gene maps to either chromosome 14 or 6
(SSC14 or 6) .

Table 4

Character	Chr.	Position (cM)	F-ratio	F-probability	Predicted QTL variance	Trait s.d.	Male additive effect	s.e.	Male dominance effect	s.e.
Boar flavour in lean	14	26	4.45	0.01314	8.50%	1.847	-0.578	0.236	0.704	0.38
Boar flavour in fat	14	30	5.68	0.00413	9.80%	2.132	-0.778	0.261	0.75	0.405
Skatole (sensory panel)	14	31	7.65	0.00067	11.40%	2.368	-1.097	0.282	0.381	0.432
Log skatole (lab)	14	46	7.74	0.00062	11.40%	0.471	-0.212	0.059	-0.105	0.089
Log indole (lab)	14	46	6	0.00306	8.90%	0.436	-0.168	0.055	-0.108	0.083
Log indole+skatole (lab)	14	47	8.43	0.00033	12.30%	0.409	-0.192	0.051	-0.093	0.077

1 CLAIMS

2

3 1. A method for determining whether a pig is
4 predisposed to boar taint comprising assaying
5 for the presence of alleles conveying
6 susceptibility to boar taint using genetic
7 markers selected from the group SW1057, SW782,
8 SW1057, S0121, SW322 or regions of chromosome 6
9 spanning therebetween.

10

11 2. A method for determining whether a pig is
12 predisposed to boar taint comprising an assay
13 for the presence of alleles conveying
14 susceptibility to boar taint using genetic
15 markers selected from the group SW857, SW2496,
16 SW295, SW210, S0007, SW761, SW1557 or regions of
17 chromosome 14 spanning therebetween.

18

19 3. An assay to identify pigs with a genetic
20 predisposition that reduces the incidence of
21 boar taint, wherein said assay comprises;
22 obtaining a DNA sample from a test pig,
23 analysing said sample to determine the
24 allelic variants present at least one genetic
25 marker, wherein said marker is selected from;
26 SW1057, SW782, SW1057, S0121, SW322 or
27 regions of chromosome 6 spanning therebetween;
28 and SW857, SW2496, SW295, SW210, S0007,
29 SW761, SW1557 or regions of chromosome 14
30 spanning therebetween;

1 and using the genotypic data from said
2 marker(s) to select for pigs of preferred
3 genotype.

5 4. A method of identifying boars which have a
6 genetic disposition to reduce boar taint, said
7 method comprising obtaining a DNA sample from
8 said boar, and
9 assaying said boar for genotypes for at
10 least one of the genetic markers identified in
11 claim 3.

13 5. A method to identify pigs with a genetic
14 predisposition which reduces the incidence of
15 boar taint wherein said method comprises;
16 obtaining DNA samples from a population of
17 pigs;
18 genotyping at least a sample of said
19 population for pre-determined markers that map
20 within or close to the QTL for boar taint traits
21 on chromosomes 6 and 14, using markers referred
22 to above or other markers located on either of
23 chromosomes 6 and 14 at a location displaying a
24 high F ratio;
25 measuring boar taint traits for at least a
26 sample of said population;
27 correlating the presence of allelic
28 variants of said markers with said traits;
29 obtaining a DNA sample from a test pig;

1 analysing the sample to determine the
2 allelic variant(s) present at a said genetic
3 marker; and

4 using said marker results to select for
5 pigs of the preferred genotype.

6
7 6. A method of identifying boars which are
8 genetically predisposed for reduced boar taint,
9 comprising obtaining a DNA sample from said boar
10 and assaying said sample for genetic variants in
11 the CYP2E gene on chromosome 6 or 14 or in the
12 region of the genome linked to this gene.

13
14 7. A method of detecting the predisposition to boar
15 taint comprising the detection of genes located
16 between the positions of the genetic markers
17 SW1057 and SW322 on chromosome 6, variation in
18 which can influence boar taint or its component
19 traits.

20
21 8. A method of detecting the predisposition to boar
22 taint comprising the detection of genes located
23 between the position of the genetic markers
24 SW857 and SW1557 on chromosome 14, variation in
25 which can influence boar taint or its component
26 traits.

27
28 9. A method of detecting the predisposition to boar
29 taint comprising the detection of markers
30 located between the positions of the genetic
31 amrkers SW1057 and SW322 on chromosome 6,

1 variation in which can influence boar taint or
2 its component traits.

3

4 10. A method of detecting the predisposition to boar
5 taint comprising the detection of markers
6 located between the position of the genetic
7 markers SW857 and SW1557 on chromosome 14,
8 variation in which can influence boar taint or
9 its component traits.

Figure 1. Chromosome 6, laboratory taint measures

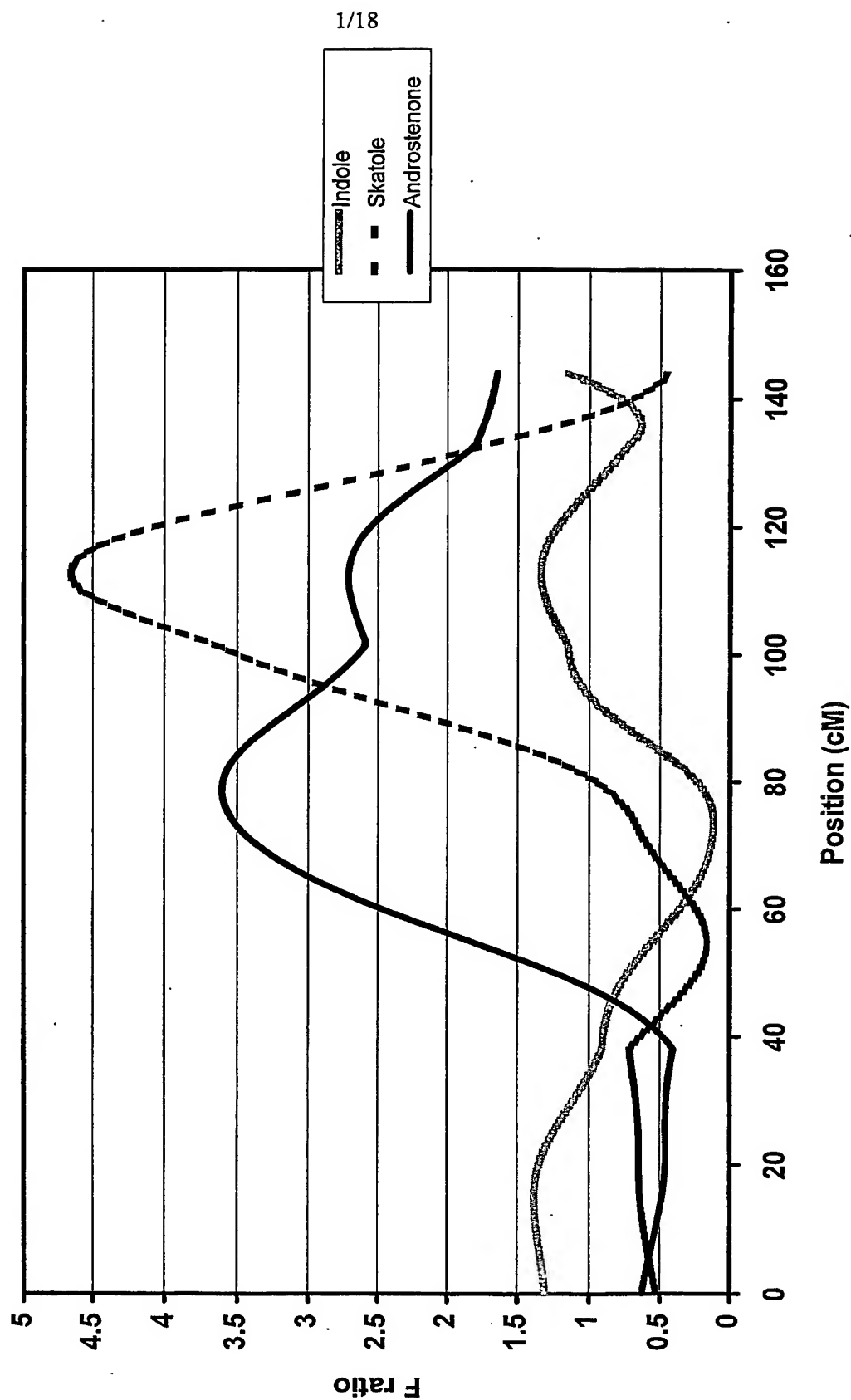
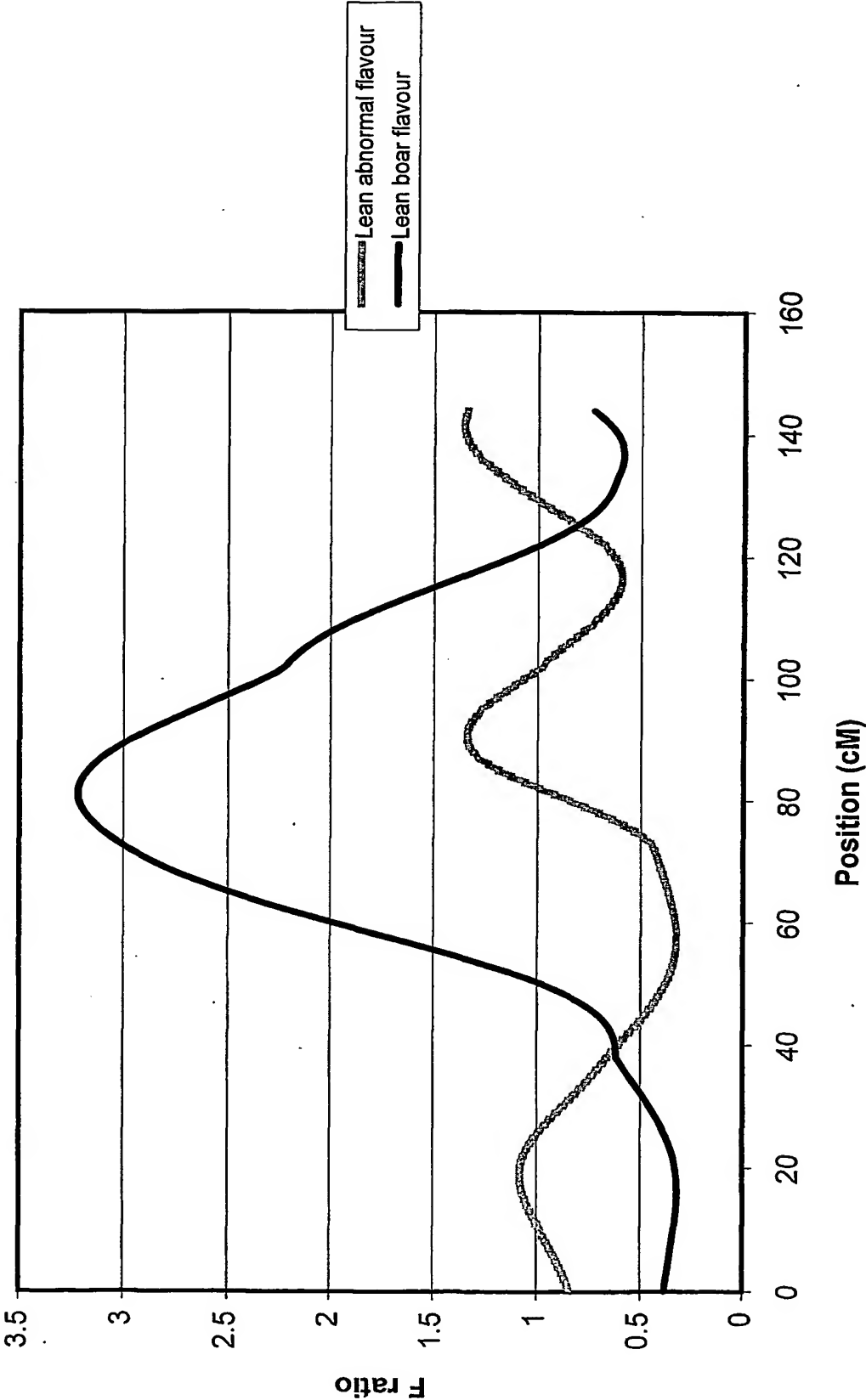


Figure 3. Chromosome 6, taste traits



3/18

Figure 2. Chromosome 14, laboratory taint measures

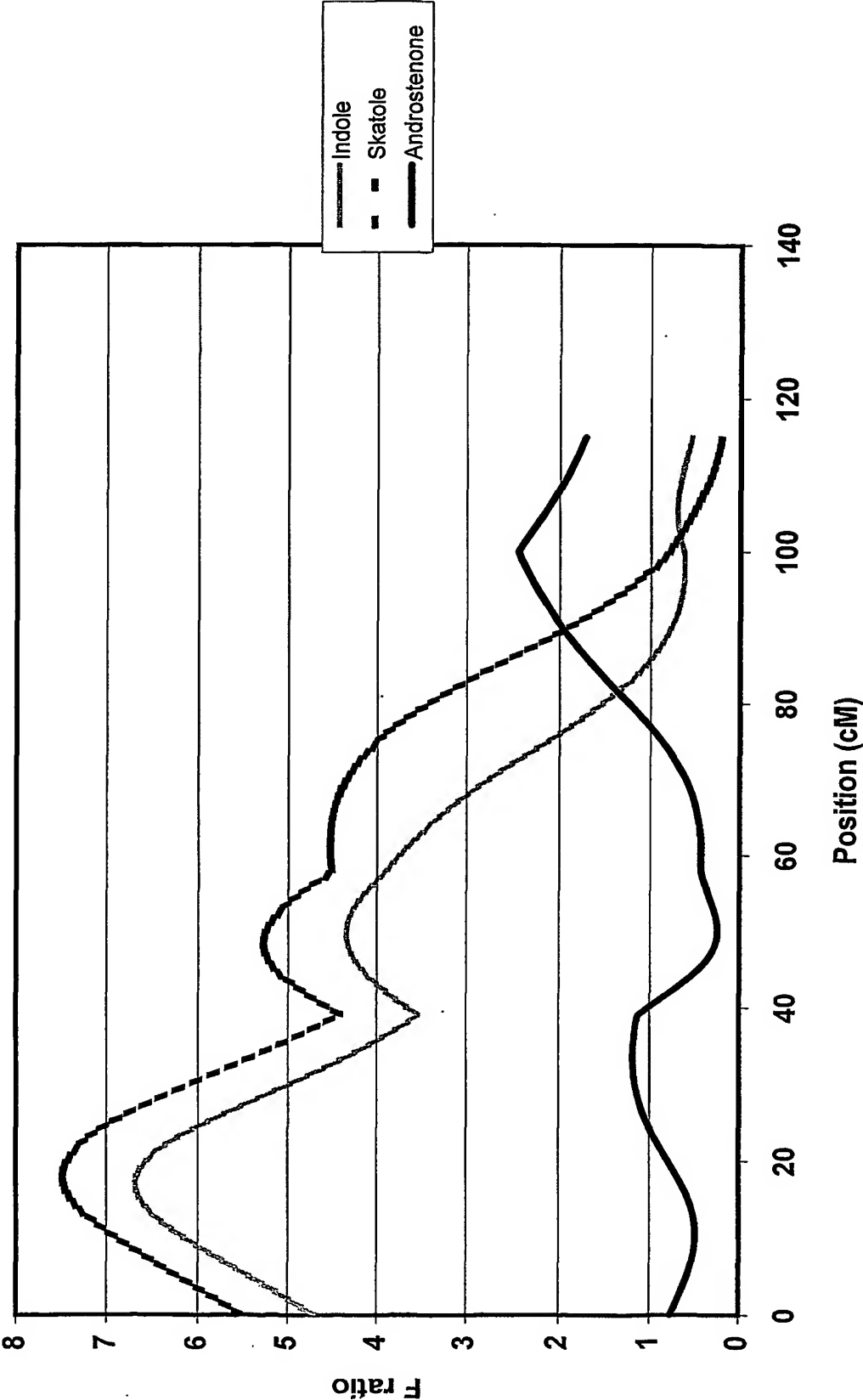
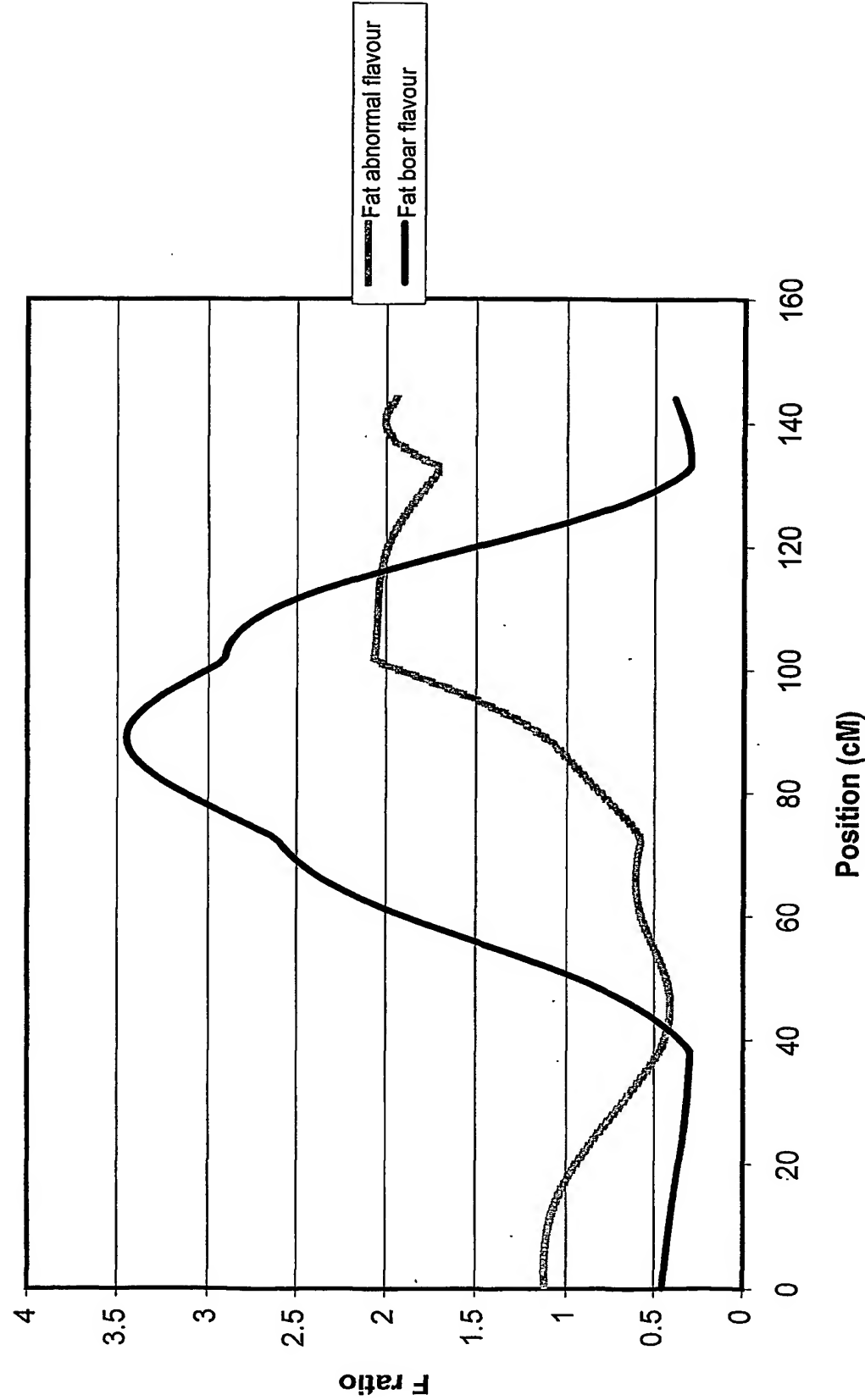
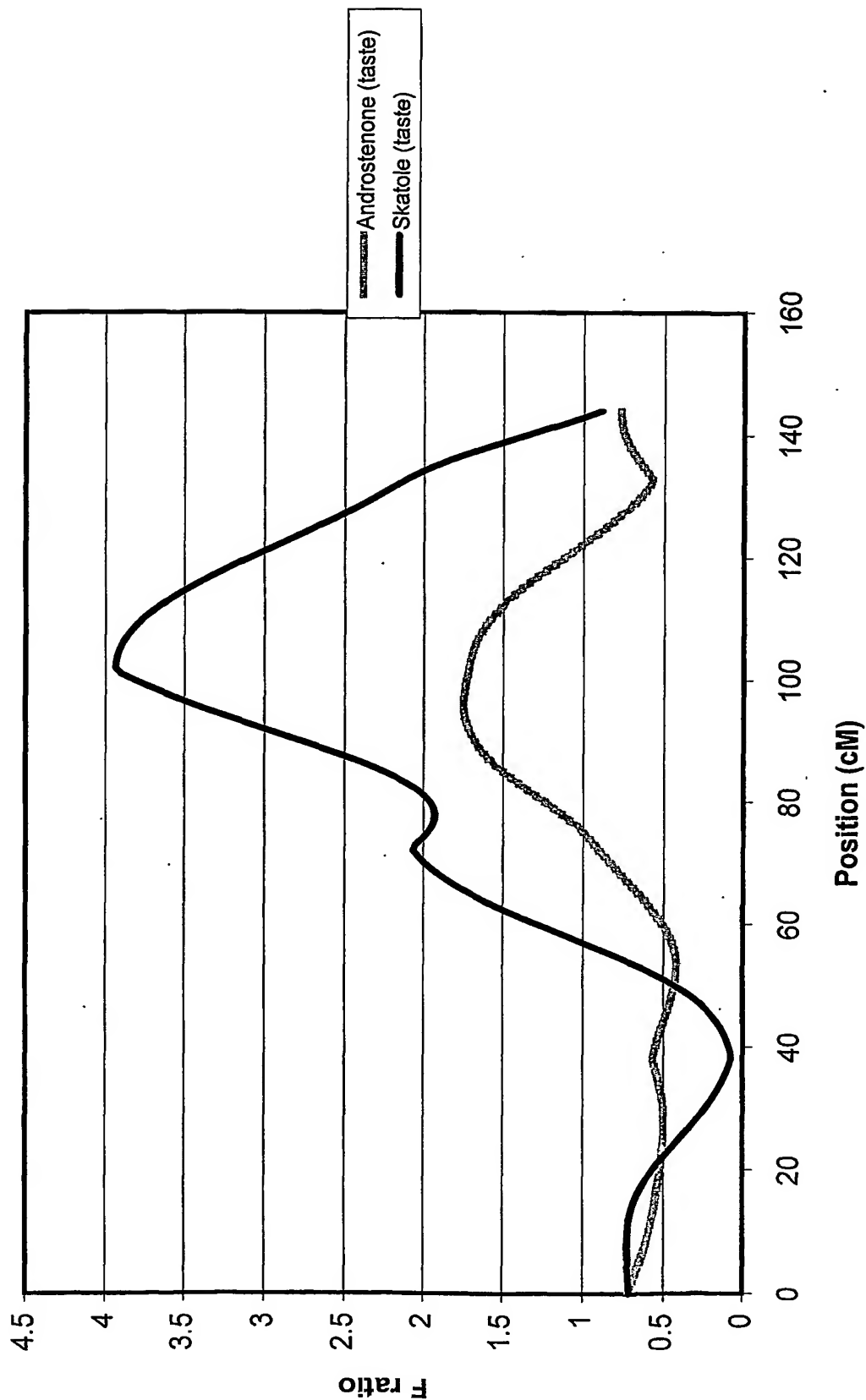


Figure 4. Chromosome 6, taste traits



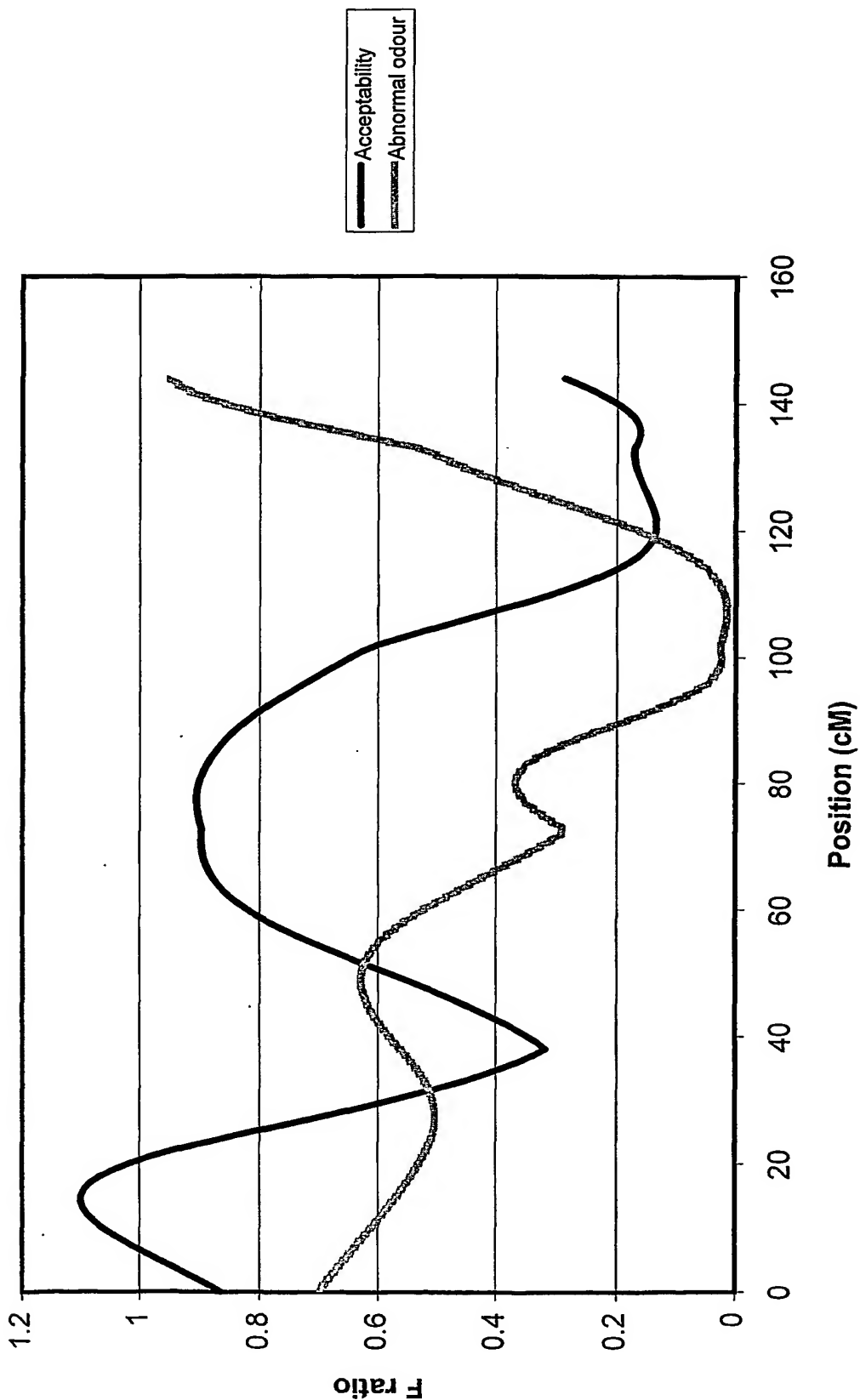
5/18

Figure 5. Chromosome 6, taste traits



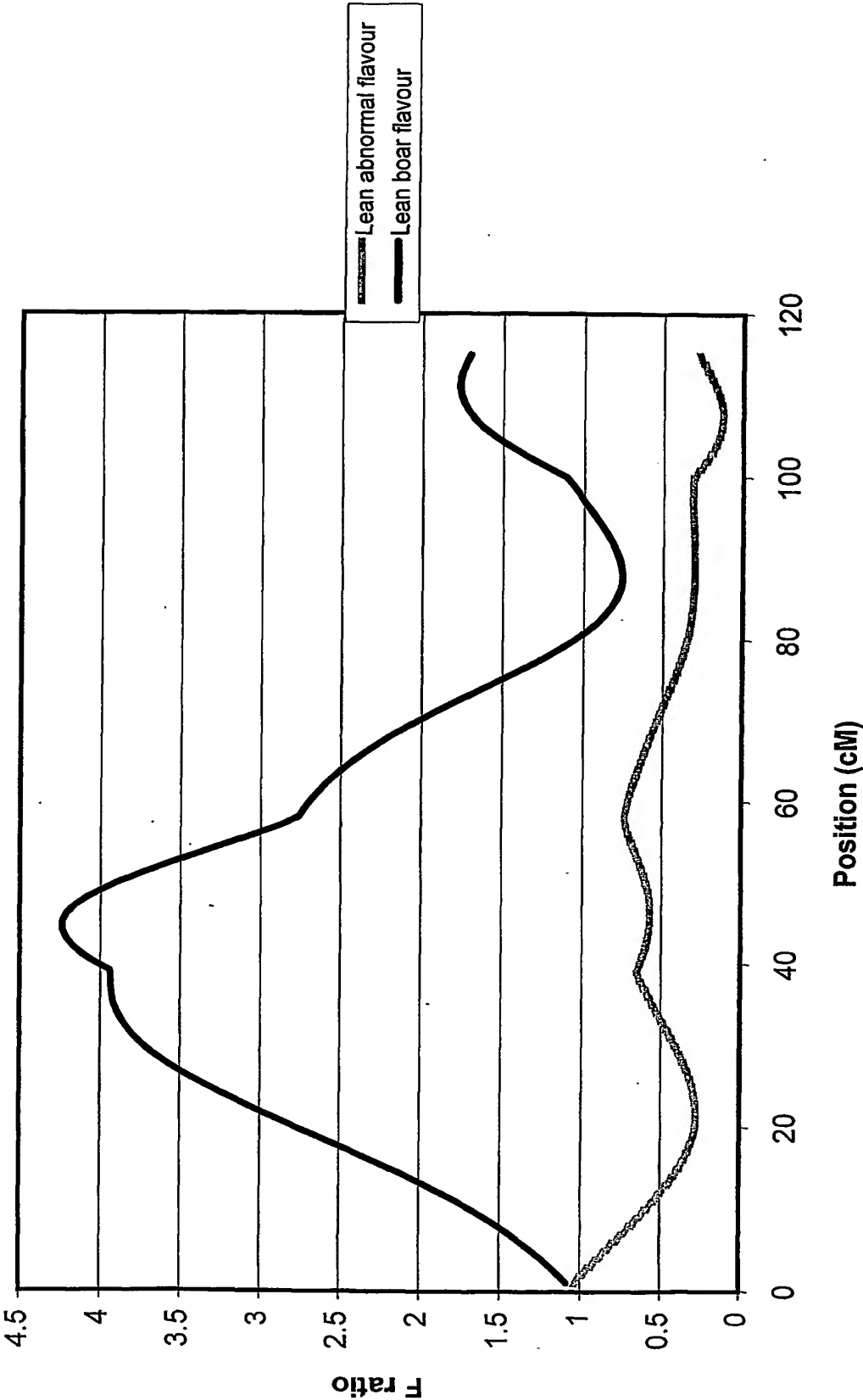
6/18

Figure 6. Chromosome 6, taste traits



7/18

Figure 7. Chromosome 14, taste traits



8/18

Figure 8. Chromosome 14, taste traits

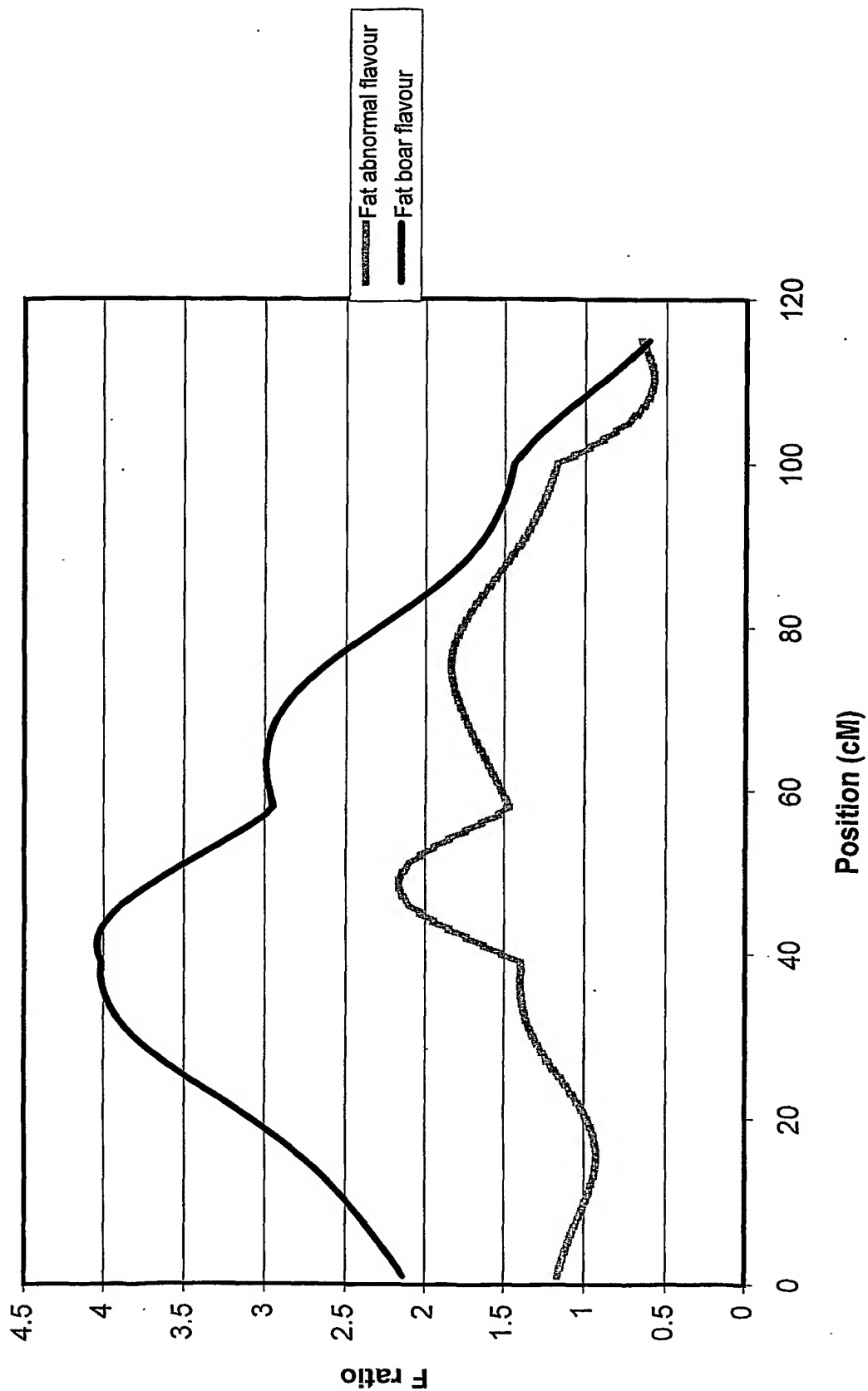


Figure 9. Chromosome 14, taste traits

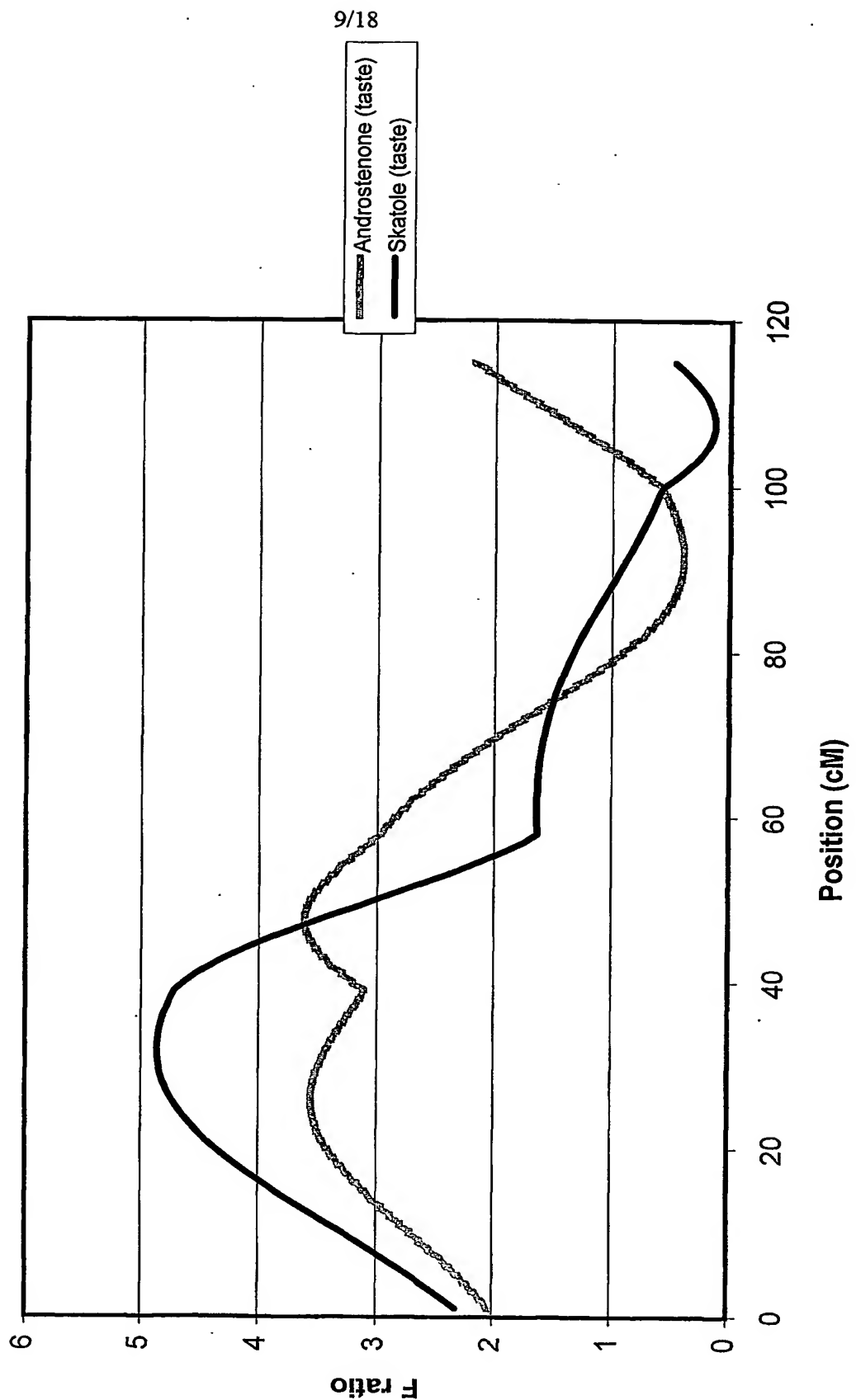
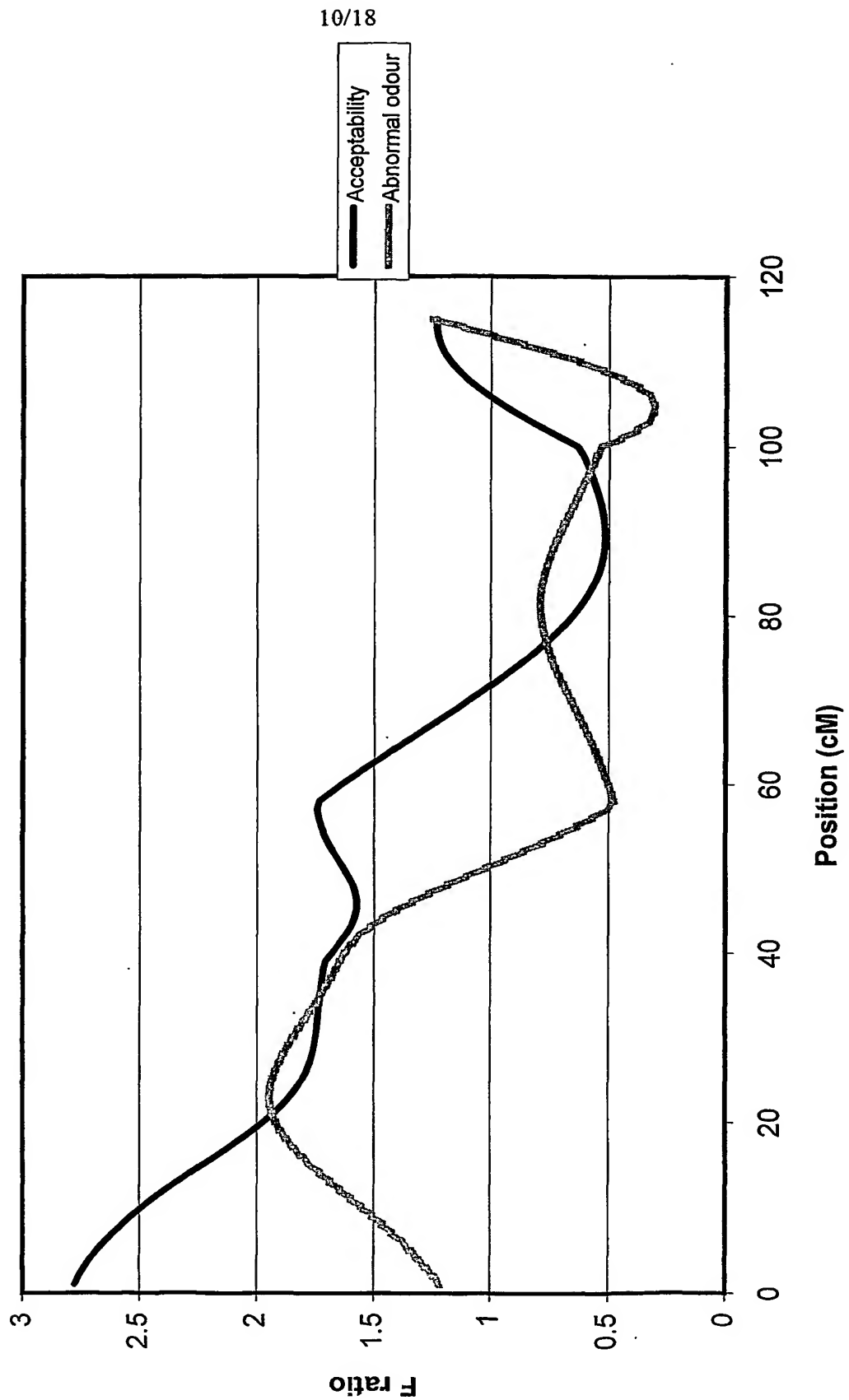
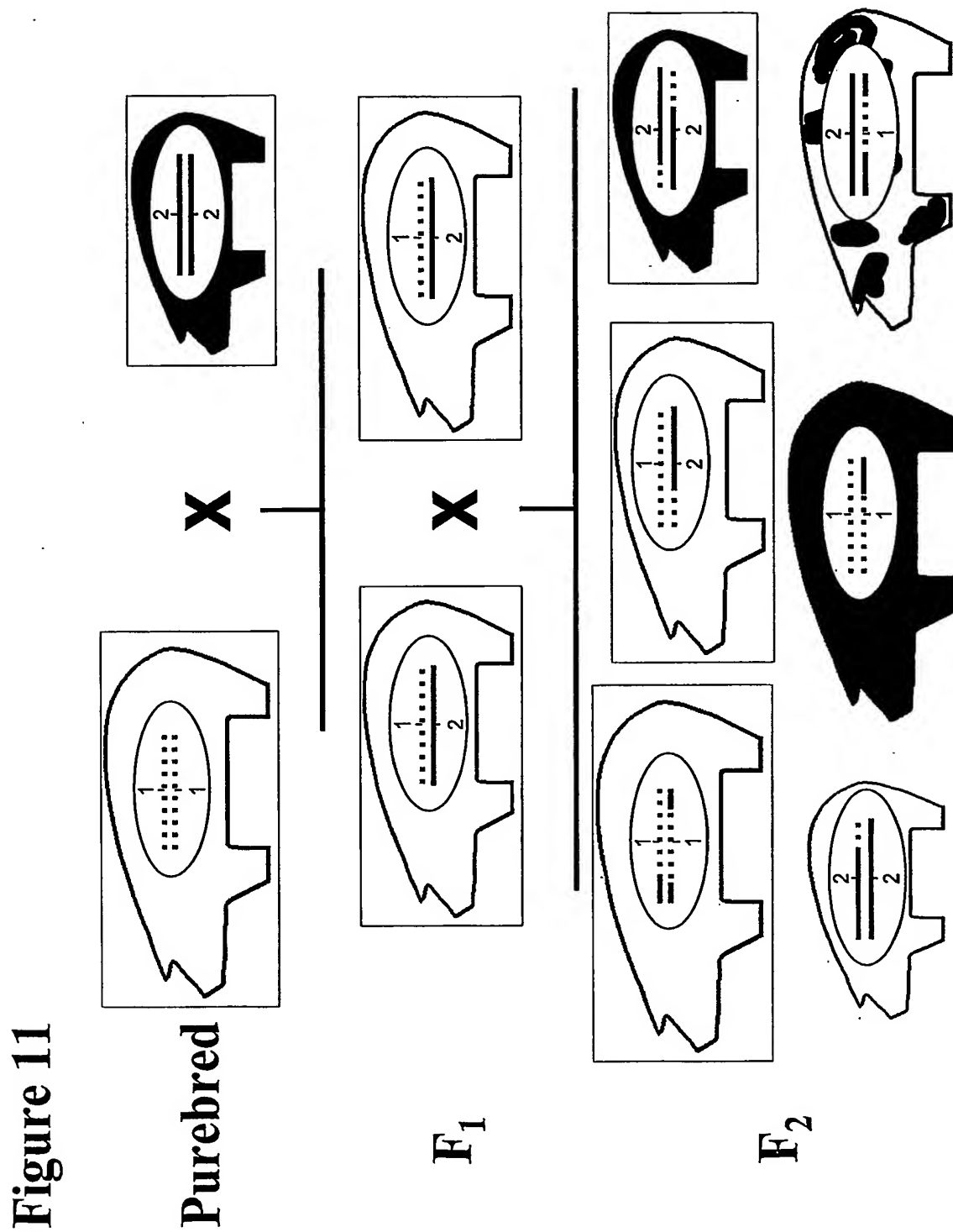


Figure 10. Chromosome 14, taste traits

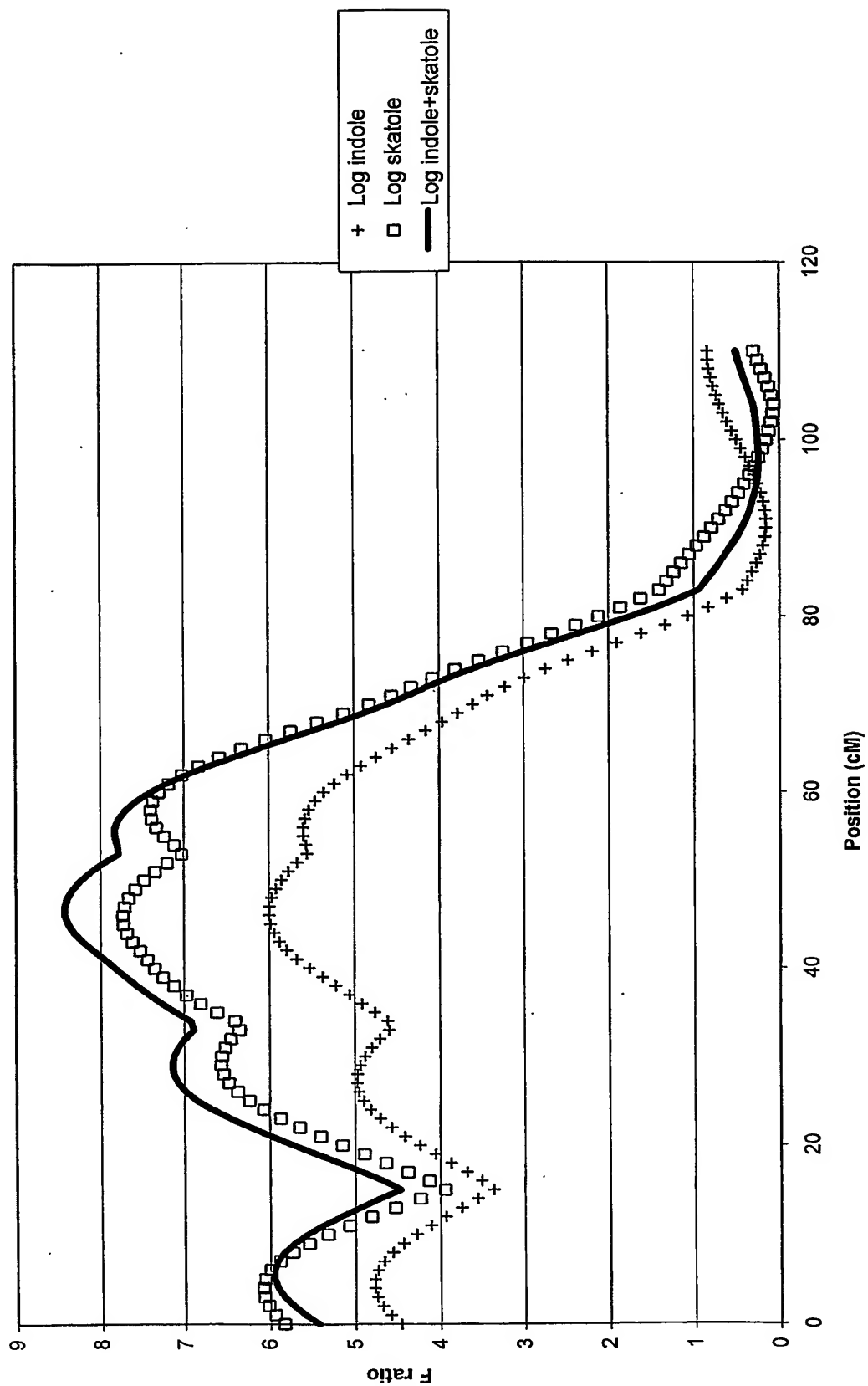


11/18



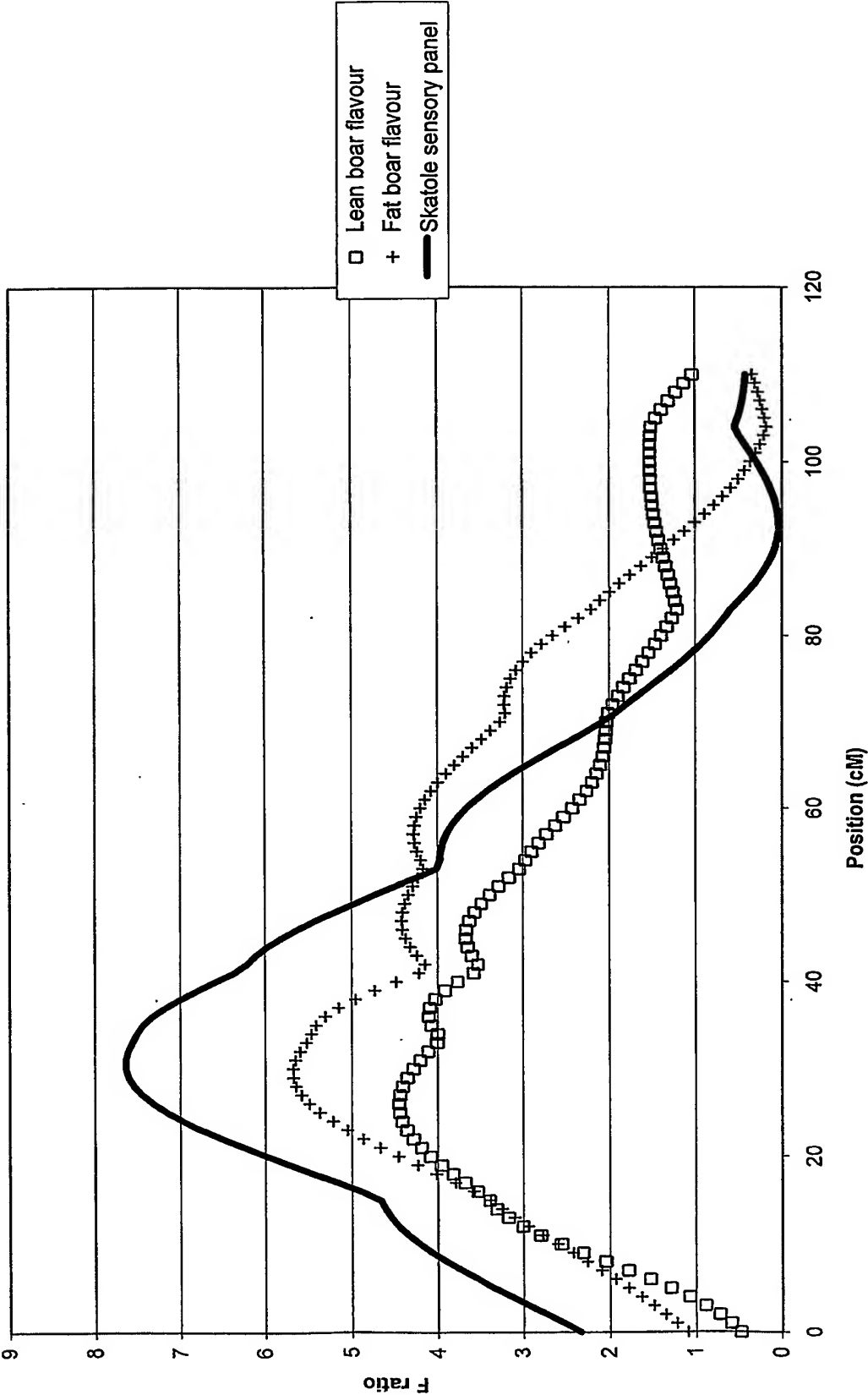
12/18

Figure 12. Chromosome 14 - 9 marker analyses, laboratory measures



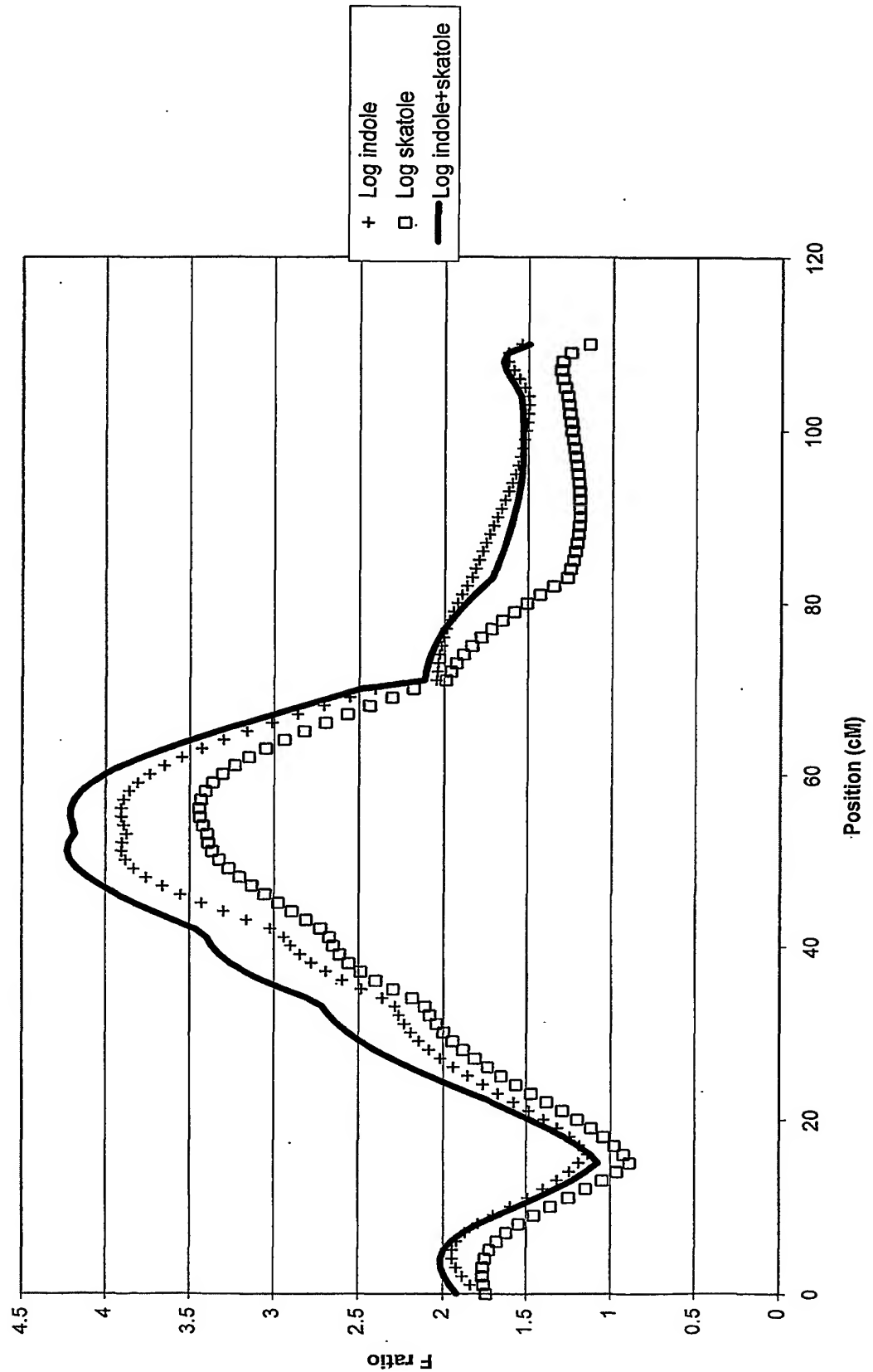
13/18

Figure 13. Chromosome 14 - 9 marker analyses, sensory panel measures



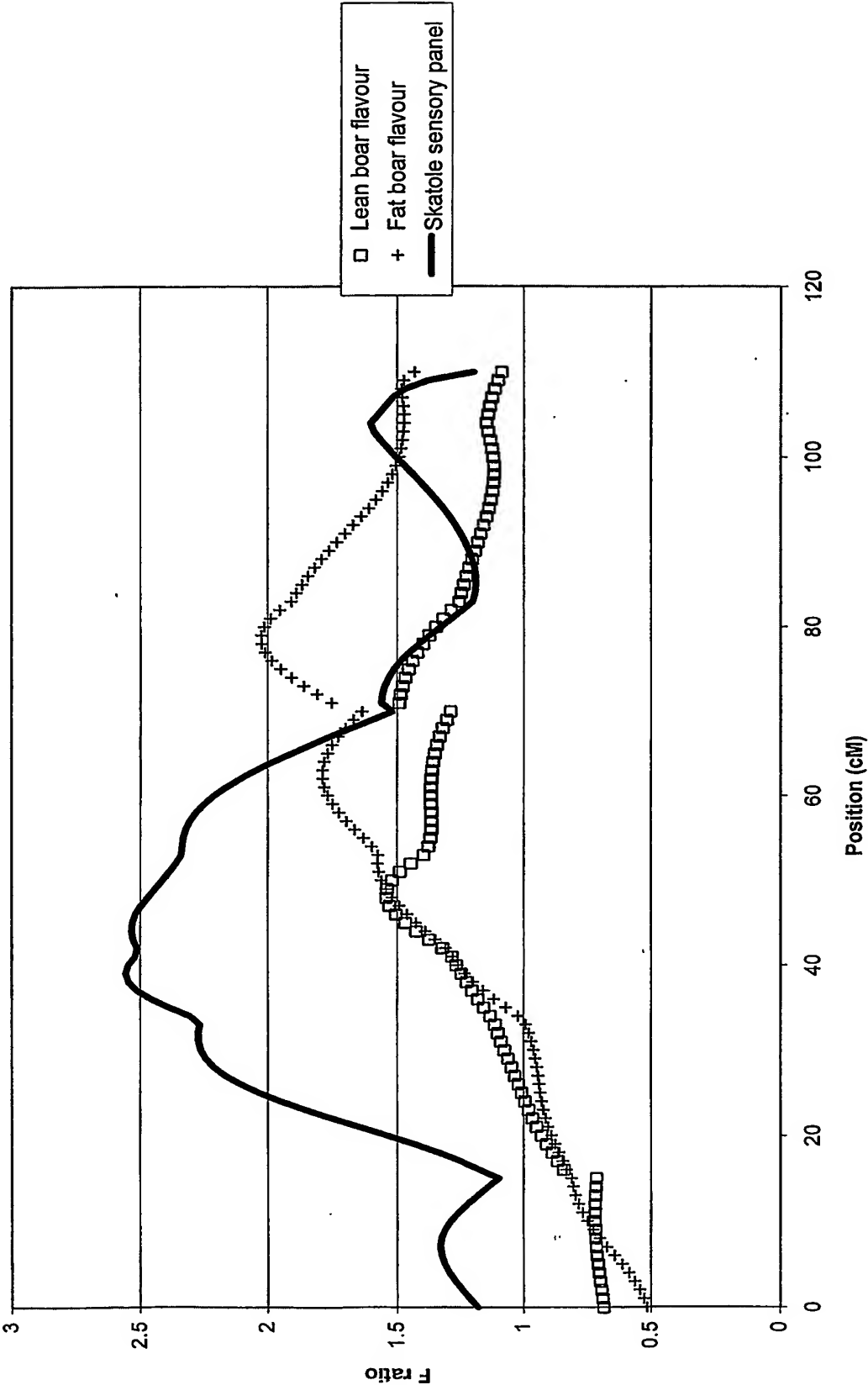
14/18

Figure 14. Chromosome 14 - 9 marker analyses with sire interaction, laboratory measures



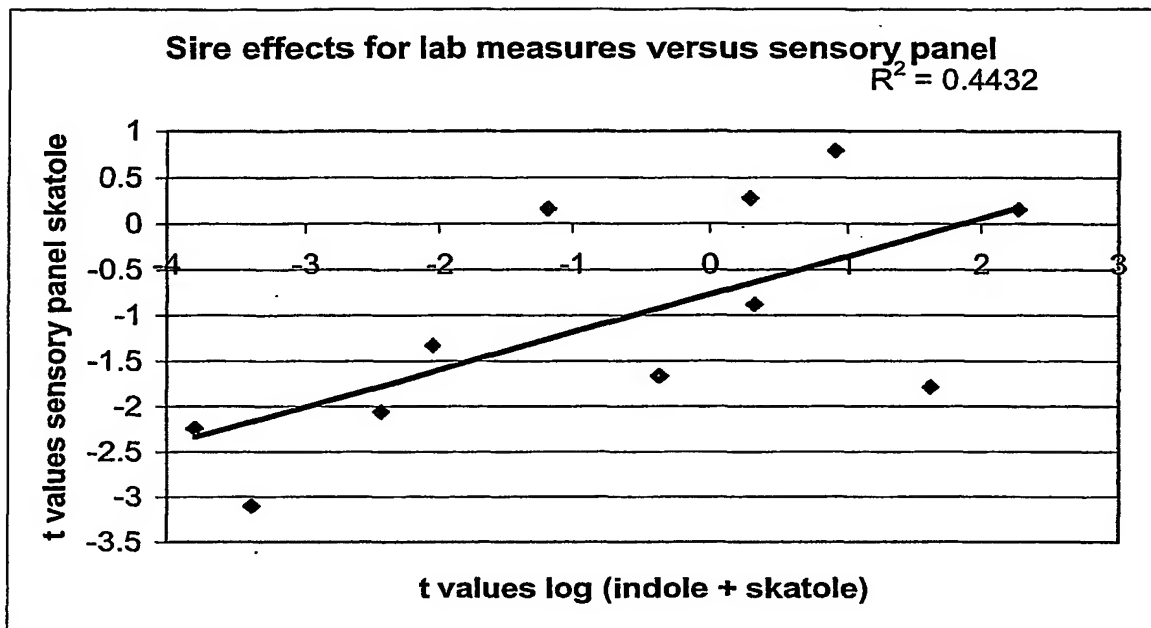
15/18

Figure 15. Chromosome 14 - 9 marker analyses with sire interaction, sensory panel measures



16/18

Figure 16



17/18

Table 3a

Character	Chromosome	Position (cM)	F-ratio	F - Probability	Predicted QTL variance% (male)	Predicted QTL variance% (female)
Boar flavour in lean	6	81	3.22	0.01314	16.17	6.24
Boar flavour in fat	6	89	3.45	0.00896	17.93	4.67
Skatole (taste panel)	6	102	3.94	0.00394	13.66	0.05
Log androstenone (lab)	6	79	3.61	0.00686	9.14	11.42
Log skatole (lab)	6	113	4.66	0.00117	30.76	0.14
Skatole (taste panel)	14	32	4.86	0.00083	18.5	0.03
Boar flavour in fat	14	41	4.04	0.00333	13.61	0.13
Boar flavour in lean	14	44	4.24	0.00237	14.14	1.97
Androstenone (taste panel)	14	47	3.62	0.00675	13.26	1.22
Log indole (lab)	14	17	6.69	0.00004	34.42	1.03
Log skatole (lab)	14	18	7.48	0.00001	30.28	6.11
Log androstenone (lab)	14	100	2.45	0.04635	1.94	10.79

Table 3b

Character	Ch.	Trait s.d.	Male additive effect	s.e	Male dominance effect	s.e.	Female additive effect	s.e.	Female dominance effect	s.e.
Boar flavour in lean	6	1.59E+00	2.94E-01	2.45E-01	-1.15 E+00	4.43E-01	3.16E-01	3.00E-01	-6.57E-01	5.00E-01
Boar flavour in fat	6	1.77E+00	6.84E-01	2.69E-01	-1.14 E+00	4.85E-01	2.11E-01	3.23E-01	-7.04E-01	5.26E-01
Skatole (taste panel)	6	1.93E+00	8.12E-01	2.38E-01	-8.51 E-01	3.54E-01	-7.40E-03	2.96E-01	8.80E-02	4.50E-01
Log androsthenone (lab)	6	1.21E+00	4.80E-01	1.84E-01	-2.79 E-01	3.25E-01	-5.76E-01	2.26E-01	-9.37E-02	3.75E-01
Log skatole (lab)	6	9.23E-01	1.25E-01	1.32E-01	-1.01 E+00	2.38E-01 2.84E-02	2.84E-02	1.65E-01	5.65E-02	2.72E-01
Skatole (taste panel)	14	1.93E+00	-1.12E+00	2.60E-01	5.01 E-01	4.57E-01	9.99E-04	3.12E-01	6.47E-02	5.20E-01
Boar flavour in fat	14	1.77E+00	-7.60E-01	2.17E-01	7.38 E-01	3.38E-01	-3.55E-03	2.62E-01	1.25E-01	3.98E-01
Boar flavour in lean	14	1.59E+00	-6.41E-01	2.02E-01	7.83 E-01	3.25E-01	3.16E-01	2.48E-01	1.33E-02	3.95E-01
Androsthenone (taste panel)	14	1.33E+00	-5.44E-01	1.72E-01	5.86 E-01	2.78E-01	9.40E-02	2.12E-01	-2.62E-01	3.49E-01
Log indole (lab)	14	8.59E-01	-5.86E-01	1.27E-01	-5.73 E-01	2.50E-01	-5.55E-02	1.52E-01	-1.56E-01	2.88E-01
Log skatole (lab)	14	9.23E-01	-7.04E-01	1.36E-01	-2.05 E-01	2.70E-01	-1.85E-01	1.63E-01	-3.74E-01	3.11E-01
Log androsthenone (lab)	14	1.21E+00	-1.95E-01	1.61E-01	1.96 E-01	2.64E-01	5.19E-01	1.97E-01	3.10E-01	3.30E-01